Kodak

November 27, 2006

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Washington, DC 20004-0001

ATTN: 8(d) Health and Safety Reporting Rule

Subject: CAS # 4170-30-3

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Studies submitted by: Eastman Kodak Company
343 State Street
Rochester, NY 14650
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Eastman Kodak Company (Kodak) is submitting the following study (studies) in accordance with EPA's final rule on August 16, 2006, requiring submission of unpublished health and safety studies. To assist EPA, Kodak elected to submit all studies that met the reporting criteria, regardless of Kodak's manufacture or import history.

Study name	Report Date	Results
Acute and chronic toxicity to daphnia magna	11/24/1992	Acute LOEC = 2.5 mg A.I./L; Chronic NOEC = 1.5 mg A.I./L; Geometric Mean MATC = 1.9 mg A.I./L; Acute/Chronic Ratio = 1
Toxicity to pimephales promelas	11/24/1992	Mean larval length was the most sensitive indicator of toxicity. The LOEC = 0.22 mg A.I./L; biological significance uncertain, thus LOEC should be considered conservative. NOEC = 0.11 mg A.I./L. Geometric Mean MATC = 0.16 mg A.I./L.

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Sincerely,

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CJV:JVN

Enc (2)

File: TSCA 8(d) Report - 2006

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STUDY TITLE

CROTONALDEHYDE - THE CHRONIC TOXICITY TO *Daphnia magna* UNDER FLOW-THROUGH CONDITIONS

(In Accordance with Guideline #797.1330)

HAEL No. 92-0072 KAN 901878 CAS No. 4170-30-3

FINAL REPORT

AUTHOR

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PERFORMING LABORATORY

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Wareham, MA 02571

LABORATORY PROJECT ID

SLI Report # 92-10-4473

SLI Study #1852.0692.6103.130

STUDY SPONSOR:

Eastman Kodak Company

STUDY COMPLETION DATE

24 November 1992

QUALITY ASSURANCE STATEMENT

The raw data and report for **"Crotonaldehyde - The Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions"** were inspected by the Springborn Laboratories, Inc., Environmental Sciences Division, Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. Dates of study inspections and dates reported to the Study Director and to Management are provided below.

It is the opinion of the QAU that this report accurately reflects the raw data collected during this study.

Inspection Date	Phase(s) Inspected	Reported to Study Director	Reported to Management
9/11/92	counting young	9/11/91	9/11/92
9/14/92	water quality measurements	9/14/92	9/25/92
10/9/92	raw data	10/9/92	10/9/92
10/22/92	raw data	10/23/92	10/23/92
10/27/92	raw data	10/27/92	11/6/92
11/2/92	raw data	11/2/92	11/6/92
11/2/92	draft report	11/2/92	11/6/92
11/23/92	final report	11/23/92	11/24/92
11/24/92	final report	11/24/92	11/24/92

SPRINGBORN LABORATORIES, INC.

Patricia D. Royal 11/24/92
Patricia D. Royal Date
Manager, Regulatory Affairs
and Quality Assurance Unit

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

To the best of my knowledge and belief, this study was conducted according to : Good Laboratory Practice Regulations for Nonclinical Laboratory Studies as promulgated by the Environmental Protection Agency Good Laboratory Practice Standard 40 CFR, Part 792, November 29, 1983 (revised August 17, 1989); with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. In addition, analyses of the dilution water used during this study for total suspended solids concentration, chlorine residue concentration, total organic carbon concentration and chemical oxygen demand concentration were also performed by Lancaster Laboratories. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization and verification of the test article identity and maintenance of records on the test article are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test article will be sent to the Study Sponsor. Archival of a sample of the test article is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

Arthur E. Putt — Nov. 24, 1992
Arthur E. Putt — Date
Study Director

EASTMAN KODAK COMPANY

Joseph W. Gorsuch — Nov. 25, 1992
Joseph W. Gorsuch — Date
Sponsor's Representative

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STUDY TITLE

Crotonaldehyde - The Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions

ABSTRACT

The purpose of this study was to estimate the chronic toxicity of crotonaldehyde to daphnids (*Daphnia magna*) under flow-through conditions. Forty *Daphnia magna* (four replicate vessels, ten daphnids per vessel) were continuously exposed for 28 days to each of five nominal concentrations of crotonaldehyde (1.5, 0.76, 0.38, 0.19 and 0.095 mg A.I./L) and a dilution water control. Observations on parental organism survival, reproduction and the number of immobilized offspring were recorded during the exposure to determine the effects of crotonaldehyde on these standard criteria.

Analyses of test article stock solutions were performed at the time of stock preparation on days 0, 7, 14, 21 and 28. Results of these analyses established that the stock solutions used to prepare the exposure solutions generally contained concentrations of crotonaldehyde at the expected concentration range of 17 mg A.I./L. Reported results are based on nominal concentrations.

During 28 days of exposure, the control daphnids survived and reproduced at rates which exceeded the minimum standard criteria in the U.S. EPA TSCA Guidelines (i.e., ≥ 80% survival, ≥ 60 offspring per female in 21 days). Following 21 days of exposure, daphnid survival and reproduction were evaluated. There was no statistically significant chemical effects at any exposure level, however, the reproduction data for the highest treatment level suggested the slight possibility of an effect. Therefore, it was decided to extend the study an additional 7 days in an effort to establish if a significant effect could be confirmed at the higher exposure concentration. At test termination (day 28), daphnid survival in the concentrations tested (1.5 - 0.095 mg A.I./L) ranged from 85 -100%. Statistical analysis (Dunnett's Test) established that survival among organisms exposed to the highest treatment level (1.5 mg A.I./L) was not statistically different from the performance of control organisms

(98%). Since no concentration tested elicited greater than 50% immobilization, the 28-day EC50 for this study was empirically estimated to be >1.5 mg A.I./L, the highest nominal exposure concentration.

Reproduction, determined as the mean number of offspring per female at test termination (day 28), ranged from 292 - 329 offspring/female among daphnids exposed to crotonaldehyde (1.5 - 0.095 mg A.I./L). Statistical analysis (Dunnett's procedure) established that the reproductive performance of organisms (317 offspring/female) in the highest treatment level (1.5 mg A.I./L) was comparable to the performance (345 offspring/female) of the organisms in the control solutions. Based on the absence of a statistically determined adverse effect in daphnid reproduction or survival at the highest treatment level tested, it was established that concentrations of ≤1.5 mg A.I./L crotonaldehyde were not chronically toxic to *D. magna*.

An acute flow-through study was performed by Eastman Kodak Company (Study No. EN-407-901878-1), exposing daphnids to nominal treatment levels of 10, 5, 2.5, 1.2 and 0.6 mg A.I./L crotonaldehyde. Based on data obtained during this study, the 48-hour EC50 (95% confidence intervals) was calculated to be 2.0 (1.3 - 2.5) mg/L. The Lowest-Observed-Effect Concentration was statistically determined to be 2.5 mg A.I./L.

Based on the results of the acute and chronic studies conducted at Eastman Kodak Company and Springborn Laboratories, respectively, it was established that acute exposure of crotonaldehyde concentrations ≥2.5 mg A.I./L adversely affect the survival of *D. magna*. Chronic exposure to levels less than those concentrations which elicited an adverse response during an acute exposure, did not adversely affect the organisms survival or reproductive performance. Therefore, utilizing the Lowest-Observed-Effect Concentration (LOEC) determined during the acute study (i.e., 2.5 mg A.I./L) and No-Observed-Effect Concentration (NOEC) determined during the chronic exposure (i.e., 1.5 mg A.I./L), the Maximum Acceptable Toxicant Concentration (MATC) for crotonaldehyde and *D. magna* was estimated as >1.5 mg A.I./L and <2.5 mg A.I./L (Geometric Mean MATC = 1.9 mg A.I./L). The

Acute/Chronic Ratio (ACR) for crotonaldehyde and *D. magna* (i.e., 48-hour EC50, divided by the estimated Geometric Mean MATC; 2.0/1.9), was calculated to be 1.

TESTING FACILITY

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SPONSOR

Eastman Kodak Company
Rochester, New York 14652-3617 U.S.A.

DATE OF STUDY INITIATION 28 July 1992

DATES OF CHEMICAL EXPOSURE 24 August - 21 September 1992

DATE OF STUDY COMPLETION 24 November 1992

STUDY PARTICIPANTS

Arthur E. Putt	Study Director
Mark J. Brown	Principal Investigator
Rex Tien	Analytical Chemist
Susan P. Shepherd	Coordinator, Data Management and Reporting Unit

1.0 INTRODUCTION

1.1 Objective

The objective of this study was to determine the chronic effects of crotonaldehyde on the survival and reproduction of the daphnid (*Daphnia magna*). The chronic study was performed under continuous exposure, flow-through conditions for a period of 28 days (one generation). Observations on parental organism survival, reproduction and numbers of immobilized young were recorded during the exposure to determine the effects of the test article on these standard criteria. During the chronic exposure, stock solution concentrations were analytically confirmed on days 0, 7, 14, 21 and 28. The results of this study were used to estimate the MATC, presented as the concentration range encompassing the highest concentration that had no significant ($p \leq 0.05$) effect on the test organism performance and the lowest concentration that significantly affected the exposed organisms. The MATC is expressed as the geometric mean of the lowest effect and highest No-Observed-Effect concentrations and is estimated from the most sensitive of the criteria used (e.g. adult survival, cumulative number of offspring per female produced).

1.2 Rationale

Chronic toxicity tests with freshwater invertebrates, particularly representative fish food organisms like daphnids, are often used to evaluate the toxicological properties of pesticides and other organic chemicals. Species such as *Daphnia magna* are valued as indicator organisms to evaluate effects on survival as well as possible effects on the reproduction of aquatic organisms (Biesinger *et al.*, 1974; Macek *et al.*, 1976a; Macek *et al.*, 1976b; Maki and Johnson, 1975; Nebeker and Puglisi, 1974; Schober and Lampert, 1977; Winner and Farrell, 1976). Daphnids have also been shown to be sensitive organisms for indicating the toxicity of a wide variety of test substances. Kenaga (1978) derived comparisons between test organism sensitivity using 75 insecticides and herbicides and concluded that daphnids and mysid shrimp were the most sensitive forms of test organisms among the aquatic invertebrates, birds, rats, fish and honeybees tested. This sensitivity, in combination with the daphnids small size, ease of culture and relatively short life-cycle, has established the 21-day

flow-through test as a standard for evaluating the potential chronic effects of chemicals on aquatic invertebrates.

2.0 TEST ARTICLE

Identity: Crotonaldehyde (MSDS, Appendix 1)

Sample Reference Identification No.: Lot #7-92, CAS #4170-30-3

Received at SLI: 23 July 1992

Physical/Chemical Properties

Purity: 93.8% active ingredient (A.I.) (Purity Determination, Appendix 2)

Composition: The test article was received as a clear liquid

Storage Conditions: Refrigerated at 4 °C

The test article was kept in a tightly sealed container and any head space was purged of air using nitrogen. This was done to minimize the potential for oxidation.

Carrier Solvent: The test article stock solutions were prepared in NANOpure® water without the use of a carrier solvent.

3.0 MATERIALS AND METHODS

3.1 Protocol

This study was conducted according to the procedures outlined in the protocol entitled "Protocol for Conducting a Flow-Through Life Cycle Toxicity Test with *Daphnia magna* Following TSCA Test Standard No. 797-1330", SLI Protocol #072292/TSCA 797.1330 DM-LC/KODAK, signed by the Study Director 28 July 1992, Protocol Amendment #1 dated 20 August 1992, Protocol Amendment #2 dated 24 September 1992 and Protocol Amendment #3 dated 28 October 1992 (Appendix 3). The procedures outlined in this protocol followed the TSCA Test Standard § 797.1330 (U.S. EPA. 1985, 1987. Toxic Substances Control Act

Test Guidelines, Federal Register 50(188), September 27, 1985. Amended, May, 1987), and conformed to the consent order established between Eastman Kodak Company and U.S. EPA entitled "Testing Consent Order, Crotonaldehyde" Docket # OPTS 42108). The 28-day test was conducted from 24 August 1992 - 21 September 1992. Testing was performed at the Environmental Sciences Division of Springborn Laboratories, Inc., (SLI), Wareham, Massachusetts.

3.2 Test Organisms

The daphnids (*Daphnia magna*) used in this toxicity test were obtained from populations cultured at Springborn Laboratories, Inc. (SLI). Daphnids were cultured under static renewal conditions (20 ± 2 ° C) in well water fortified to a total hardness of approximately 160 - 180 mg/L $CaCO_3$. Water used to culture these organisms was from the same source as the dilution water used during the chronic exposure. The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Light intensity in the culture area ranged from 34 - 44 footcandles. Daphnids were fed daily a combination of green algae (*Ankistrodesmus falcatus*) and a trout food suspension. In accordance with EPA-GLP, routine analyses were conducted on representative samples of the food sources for the presence of pesticides, PCBs and toxic metals (Appendix 4). Food sources were considered acceptable since the total concentration of pesticides measured was less than 0.3 mg/kg (ASTM, 1985). Offspring produced during the 24-hour period prior to the start of each exposure were used to initiate the study. Cumulative immobilization among daphnids used to initiate this exposure was 0% during the 48-hour period prior to test initiation. The first brood release among culture daphnids was recorded after seven days, and parental daphnids produced an average of six offspring per female per day during the two weeks prior to study initiation. Records of daphnid culture are retained in SLI's culture log entitled "Invertebrate Culture Log; *Daphnia magna*, Volume VIII" (1 January - 31 December 1992).

3.3 Test Diluent Water

Culture and test diluent water were identical and were prepared in 1900-liter batches by fortifying well water according to the formula for hard water (ASTM, 1980) and filtering it

through an Amberlite XAD-7 resin column and a carbon filter to remove any potential organic contaminants. Generally, several batches of water were prepared each week. The frequency at which the diluent water was prepared depended on the requirements of the laboratory. Fortified water was discarded if not used within 14 days of preparation. The water used for the 14 days prior to test initiation and during the definitive exposure was characterized as having a total hardness and alkalinity range as calcium carbonate ($CaCO_3$) of 160 - 170 mg/L and 110 - 120 mg/L, respectively, a pH range of 8.1 - 8.3, a temperature of 20 ± 1 °C and a specific conductivity of 500 micromhos per centimeter (μmhos/cm). Water quality parameters were measured on each batch of fortified water prior to use. In accordance with EPA-GLP, routine analyses were conducted on representative samples of the diluent water source for the presence of pesticides, PCBs and toxic metals (Appendix 4). None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed. In addition, representative samples of the diluent water source were analyzed monthly for total organic carbon (TOC) concentration. Based on the September analysis, the TOC concentration of the diluent water source was determined to be 1.2 mg/L. The results of these analyses and the ability of several daphnid species to survive and reproduce over several generations of culture in this water source and periodic scans for PCB and pesticide contamination confirmed that the diluent water was of acceptable quality. In addition, representative samples of the dilution water source used during this study were also analyzed monthly for total organic carbon (TOC) concentration, chemical oxygen demand (COD) concentration, total suspended solids (TSS) concentration, unionized ammonia concentration and residual chlorine concentration. Based on these analyses, the dilution water source was determined to have a TOC concentration within the range of 0.8 - 0.9 mg/L, a COD concentration of ≤ 7 mg/L, a TSS concentration of ≤ 4 mg/L, an unionized ammonia concentration of ≤ 0.1 mg/L and a residual chlorine concentration of < 0.05 mg/L.

3.4 Stock Solution

A study was conducted to determine the stability of crotonaldehyde in three different freshwater matrices: soft water (hardness approximately 26 mg/L as $CaCO_3$), hard reconstituted water (hardness approximately 170 mg/L as $CaCO_3$) and NANOpure® water

(ASTM Type II). Test solutions were prepared by fortifying each water type with the appropriate amount of crotonaldehyde to achieve a nominal concentration of 17 mg/mL. An aliquot of each solution was removed for analysis immediately upon fortification (hour 0). Subsequent samples were removed after 24 and 48 hours. An additional sample was removed from the test solution prepared in NANOpure® water after 96 hours. All samples were analyzed according to the method described in Appendix 5.

Results of analyses conducted at 0 and after 24 hours indicated that crotonaldehyde was stable in NANOpure® water but not stable in either soft or hard reconstituted water (?). Average measured concentrations of crotonaldehyde solutions prepared in hard reconstituted water averaged only 14% of the nominal fortified concentration at 0 hour and, were variable at 24 hour, with a mean of 13% of nominal (range; 8 - 18%). Measured concentrations in soft water averaged 75% of the nominal fortified concentration at 0 hour, but decreased to a maximum of 10% of nominal within 24 hours. Analysis of test solutions prepared in NANOpure® water at 0 hour and at 24 hours resulted in measured concentrations which averaged 87% and 85% of the nominal fortified concentration, respectively.

Analytical difficulties were experienced during the preparation and the analysis of the experimental samples removed at 48 hours. Based on the data obtained during the first 24 hours no further evaluation of the soft or hard reconstituted water matrices was conducted, however, additional samples were removed from the NANOpure® water test solution after 96 hours to confirm the stability of crotonaldehyde in this matrix. The results of these additional analyses (i.e., measured concentrations averaging 106% of nominal) at 96 hours indicate that crotonaldehyde was stable in NANOpure® water for at least 96 hours. Based on these data, all stock solutions of crotonaldehyde used to prepare exposure solutions for the effects tests were prepared in NANOpure® water.

The consent order specified that stock solution analysis was to be conducted every 7 days throughout the exposure period. A summary of the day of stock preparation, the day of stock analysis and the age of representative stock solutions is presented below.

Day of Preparation	Test Day Sampled and Analyzed	Age of Stock (days) at Time of Analysis
0	0	0
2	NA	NA
4	NA	NA
6	7	1
8	NA	NA
10	NA	NA
12	NA	NA
14	14	0
21	21	0
21	28	7

NA = Not Applicable

Table 3 presents the results of the analysis of the stock solutions for crotonaldehyde concentration performed on days 0, 7, 14, 21 and 28.

Analysis of stock solutions during the in-life portion of the *Daphnia magna* life-cycle study and the early life-stage study with fathead minnow (SLI Report #92-10-4472) further indicated that the crotonaldehyde stock solutions (prepared in NANOpure® water) were generally stable for 7 days. Although some variability is apparent in the individual measured values, the average measured concentrations resulting from analyses conducted on 7-day old stock solutions did not differ significantly relative to the nominal concentrations. Measured stock solution concentrations and diluter calibrations established that crotonaldehyde was being delivered to the exposure system at concentrations consistent with the nominal treatment levels.

Stock solutions of 17 mg A.I./mL were prepared on exposure days 0, 2, 4, 6, 8, 10 and 12 by diluting 3.652 g of test article (3.42 g active ingredient) with NANOpure® water (which had been previously purged with nitrogen for 3 - 4 minutes prior to use) to a volume of 200 mL (Chemical Distribution Record, Appendix 6). Stock solutions of 17 mg A.I./mL diluter stock was prepared on test days 14, 21 and 28 by dissolving 9.131 g of test article (8.56 g active ingredient) with NANOpure® water (also purged with nitrogen for 3 - 4 minutes) to a volume of 500 mL. Diluter stocks were observed to be clear and colorless.

3.5 Test Conditions

A 200-mL intermittent-flow proportional diluter (Mount and Brungs, 1967), calibrated to provide 50 percent dilutions between adjacent concentrations, delivered the diluent water and the crotonaldehyde to the test vessels during the chronic toxicity test. The diluter was constructed entirely of glass and silicone tubing, stoppers and sealant. The diluter system was equipped with a 50-mL gas-tight syringe on a Lirette mechanism which delivered 0.035 mL of the crotonaldehyde stock solution (17 mg A.I./mL) into 395 mL of diluent water in the system's mixing chamber during each diluter cycle. This 395-mL solution (nominal concentration of 1.5 mg A.I./L) served as the highest treatment level from which calibrated volumes were diluted to provide the 50% nominal concentration gradient (0.76 to 0.095 mg A.I./L). Four 5-centimeter (cm) lengths of 1-millimeter (inside diameter) glass capillary tubing were inserted through silicone stoppers in the mixing/splitting chambers of the diluter and into the test solution delivery tubes. This tubing served to restrict the flow of the test solutions, minimizing potentially stressful turbulence in the test vessels and providing equal distribution of the test solutions to the replicate vessels.

Test vessels were glass battery jars having a volume capacity of 1.4 liters. Test solutions drained from each vessel through a 2-cm hole, drilled approximately 15 cm from the bottom of the jar. The drains were covered with a Nitex® 40-mesh screen to prevent loss of the daphnids. Test vessels were loosely covered with plastic wrap throughout the duration of the exposure period. In addition to the five concentrations of crotonaldehyde, a diluent water control solution was maintained. All treatment levels and the control consisted of

quadruplicate vessels. The four vessels for each treatment/control were arranged in rows; the position of each vessel was randomized within each row. Test solutions were delivered to the vessels at an approximate rate of 6.0 aquarium volumes per 24-hour period in order to provide 90% test solution replacement within 9 hours (Sprague, 1969). The test area was illuminated with Durotest® Vita-Lite and Sylvania® Cool-White fluorescent lights at an intensity of 38 to 52 footcandles and a photoperiod of 16 hours of light and 8 hours of darkness. The study was conducted in a water bath designed to maintain the test solution temperatures at 20 ± 2°C.

4.0 TEST PROCEDURES

4.1 Test Initiation

Selection of crotonaldehyde concentrations for the chronic exposure was based on the results of preliminary flow-through testing conducted at SLI. At the initiation of the study, *Daphnia magna* (≤24 hours old) were impartially selected and distributed among 24 unlabeled intermediate vessels (i.e., 100 mL beakers) containing 40 mL of dilution water and several drops of algal food solution. The daphnids were impartially added, two at a time, to each intermediate vessel until all vessels contained two organisms. The process was repeated until each vessel contained ten organisms. The daphnids were then introduced into the exposure replicate vessels (starting from the control and progressing to the highest treatment level) by impartially selecting one of the unlabeled intermediate vessels containing ten organisms and gently pipetting them one at a time under the surface of the test solution. This process was repeated until each test concentration contained forty *Daphnia magna* (10 per replicate). Food solutions (5.5 mL total) were added to the exposure solutions prior to the introduction of daphnids. The test was initiated after the diluter and toxicant delivery device had been observed to be properly functioning for four days prior to test initiation. A visual check of proper diluter function was performed at least once daily throughout the study.

4.2 Test Monitoring

The number of immobilized adult daphnids and observations of abnormal behavior were recorded on days 1, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28. Assessments of offspring production were determined on day 7 and three times per week thereafter through day 28. Immobilization is defined by lack of movement by daphnids except for minor activity of appendages. Immobilization and reproduction were determined by counting and observing adults as they were carefully pipetted from the exposure vessel to a 100 mL beaker containing approximately 50 mL of the respective test solution. The 50 mL of test solution was removed from the respective test vessel by gently immersing the 100 mL beaker into the test solution and filling to the 50 mL mark. After removing the adult daphnids, the exposure solution was then filtered through a fine mesh net into a holding vessel to remove offspring. Offspring were removed from the net by inverting and immersing the net into a 100 mL beaker containing dilution water. These 100 mL beakers containing the offspring were put aside and counted after determination of adult immobilization. The exposure vessel was then cleaned and carefully rinsed with water. The original test solution was then returned and the beaker containing the adult daphnids was lowered in the exposure vessel and slowly tipped to allow the water to flow slowly into the test vessel and allow the daphnids to swim out. After each observation interval, the offspring were removed, counted and discarded. The number of immobilized offspring and the time to first brood release were recorded for each replicate vessel. In addition, observations of physical characteristics of test solutions (e.g., precipitate, cloudy solution) were recorded whenever test organisms were observed. Test vessels were washed a minimum of three times per week.

The test organisms were fed a diet consisting of a combination of trout food suspension (5 mg/mL), a suspension of green algae (*Ankistrodesmus falcatus*; 4 x 10^7 cells/mL) and Selco® (a commercial mixture of proteins and fatty acids, 0.6 mg/mL). During the exposure; the food was introduced at a rate of 2.0 mL of trout food suspension, 3.0 mL of algal suspension and 0.5 mL of Selco® food supplement three times daily on weekdays and twice daily on weekends.

4.3 Water Quality Measurements

The test solution temperature was measured daily in one replicate vessel of each treatment level and control solution throughout the 28-day exposure. In addition, the test solution temperature was continuously monitored in one replicate (A) of the 1.5 mg A.I./L solution throughout the study using a Computemp 5 (SN#95) thermometer. The dissolved oxygen concentration and pH of the test solutions were measured daily throughout the exposure period in one replicate vessel of each treatment level and the controls. The pH, dissolved oxygen concentration and temperature were measured once a week in all replicate vessels of each treatment level and the controls. The dissolved oxygen concentration was measured using a YSI Model #57 dissolved oxygen meter. A LaMotte Model HA, Hanna Model HI9024 and a Jenco 601A pH meter were used for pH measurements. Daily and weekly temperature measurements in each treatment level and the controls were made using a Brooklyn alcohol thermometer. Total hardness, alkalinity and specific conductivity of the test solutions were monitored weekly in one replicate vessel from each treatment level and the control solutions. Specific conductivity was monitored with a YSI Model #33 conductivity-salinity meter. Total hardness and alkalinity of the test solutions were determined according to APHA *et. al.* (1985). Light intensity was measured with a General Electric Type 214 light meter.

4.4 Analytical Measurements

Triplicate samples of stock solutions were analyzed on days 0, 7, 14 and 21 for crotonaldehyde concentration. All samples were analyzed within 24 hours of preparation with the exception of day 0, which was analyzed 1 day after preparation. In addition, duplicate samples of the stock solution prepared on day 21 were analyzed on day 28 in order to monitor stock stability. All samples were removed from the approximate midpoint of the volumetric flask using a volumetric pipet. Samples were derivitized and extracted immediately after sampling. Three Quality Control (QC) samples were prepared at each sampling and remained with the set of stock solution samples throughout the analytical process. These QC samples were prepared in NANOpure® water at a concentration of crotonaldehyde similar to that of the stock solution. Results of the analysis of QC samples were used to judge the

precision and quality control maintained during the analysis of the stock solution samples. All samples were analyzed utilizing a gas chromatographic (GC) procedure using the methodology presented in Appendix 5. A method validation study conducted at SLI prior to the initiation of the chronic test established a mean recovery of crotonaldehyde of 88.5 ± 5.8% from diluent water (fortified to a hardness of 160 -180 mg/L as $CaCO_3$).

5.0 STATISTICAL ANALYSES

5.1 LOEC and NOEC Determination

At the termination of the chronic study, data obtained on organism survival and reproduction were statistically analyzed to define the Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC). These levels are defined as the lowest test concentration that shows a statistically significant effect (LOEC) and the highest concentration that shows no statistically significant effect (NOEC). The following procedures were used:

1) Significant differences in the percent survival were determined after transformation (e.g., arcsine square-root percent) of the data.

2) The Shapiro-Wilks test for normality (Weber *et al.*, 1989) was conducted and compared the observed sample distribution with a normal distribution. The assumption that observations are normally distributed must be validated before subsequent analyses, following parametric procedures, can be performed. If the data are not normally distributed, then a nonparametric procedure is used for subsequent analyses.

3) As a check on the assumption of homogeneity of variance implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985). Data not meeting the assumptions of homogeneity of variance were statistically analyzed using a nonparametric method of comparison such as Kruskal-Wallis Test.

4) For this study, all parameters met assumptions for normal distribution and homogeneity of variance, and therefore, parametric statistical procedures were used to establish survival and reproductive effects. The Williams' Test (Williams, 1972, 1971) and the Dunnett's Test (Dunnett, 1955, 1964) are parametric procedures. The Williams' Test is preferred for chronic toxicity testing and is more powerful than the Dunnett's procedure (Rand and Petrocelli, 1985). However, the Williams' Test, by design, assumes a concentration-response due to increasing concentration of toxicant. When this assumption is violated, the Dunnett's procedure may be more appropriate. Since a well-defined concentration-response was not established for either parameter (survival, reproduction) during this study, Dunnett's Test was initiated to establish treatment level effects.

5) Survival data were analyzed prior to the analysis of reproduction data; any concentration that caused significant adverse effects on survival was excluded from the analysis of reproduction data.

A detailed description of each of these procedures in presented in Appendix 7.

5.2 EC50 Calculation

The concentrations tested and the corresponding biological response data (immobilization/survival) derived from the toxicity test were also used to estimate the median effect concentration (EC50) and 95% confidence interval. The EC50 is defined as the concentration of the test article in diluent water which caused immobilization of 50% of the test organism population at the stated time interval. EC50 values were estimated as being greater than the highest concentration tested since no test concentration caused 50% or more immobilization.

6.0 DATA STORAGE AND RECORDS RETENTION

All raw data and the original final report produced for this study will be stored for a minimum of ten years in the archives of the Study Sponsor. A copy of the final report will be stored in the archives of Springborn Laboratories, Inc., Wareham, Massachusetts.

7.0 RESULTS

7.1 Preliminary Testing

Prior to the performance of the definitive chronic study, a preliminary test was conducted at Springborn Laboratories, Inc. During this preliminary test, daphnids (≤ 24 hours old at initiation) were exposed, under flow-through conditions, to nominal concentrations of 750, 380, 190, 94 and 47 µg A.I./L crotonaldehyde. Following 11 days of exposure, immobilization ranging from 0 - 35% was observed in the treatment levels (750 - 47 µg A.I./L). During the same period, reproduction among daphnids exposed to the highest nominal test concentration, 750 µg A.I./L was 19 offspring/female. Reproduction in the remaining nominal test concentrations (380 - 47 µg A.I./L) ranged from 39 - 48 offspring/female. Reproduction among control daphnids averaged 40 offspring/female. An acute exposure previously conducted by Eastman Kodak Company (Study No. EN-407-901878-1) established a 48-hour EC50 of 2.0 mg/L. Based on the approximate 50% reduction of offspring produced in the 750 µg A.I./L test concentration, the following nominal concentrations were selected for the definitive chronic study: 1.5, 0.76, 0.38, 0.19 and 0.095 mg A.I./L.

7.2 Water Quality

The results of water quality determinations made during the daphnid chronic exposure demonstrate that the dissolved oxygen concentration, pH, specific conductivity, total hardness and alkalinity of the test solutions were unaffected by the concentrations of crotonaldehyde tested (Table 2). Continuous temperature monitoring in one replicate (A) of the 1.5 mg A.I./L (nominal) treatment level demonstrated that the test solution temperature ranged from 18 to 22 °C during the exposure period. Daily measurement of the temperature in each treatment level solution and control established that the average temperature was

20 °C. Water quality conditions established for the test remained within acceptable ranges for the survival, reproduction and growth of *Daphnia magna*.

7.3 Exposure Monitoring

A complete check of diluter function was made at least once daily. Diluter calibration was checked at test initiation and weekly thereafter during the study. No deviations in calibration were observed throughout the study. The diluter system which prepared and delivered the test solutions to the exposure vessels functioned properly throughout the exposure period. Throughout the 28-day study, diluter stock solutions and exposure solutions were observed to be clear and colorless. Analyses of the stock solutions for crotonaldehyde were performed on days 0, 7, 14, 21 and 28. The results of these analyses established that the stock solutions used to prepare the exposure solutions generally contained the expected concentration of crotonaldehyde (Table 3). Review of these data in addition to data collected in support of the stability study indicated that the crotonaldehyde stock solutions (prepared in NANOpure®) were generally stable for 7 days.

7.4 Biological Observations

During 28 days of exposure, the control daphnids survived and reproduced at rates which exceeded the minimum standard criteria of the U.S. EPA TSCA Guidelines (i.e., ≥ 80% survival, ≥ 60 offspring per female for 21 days of exposure). Following 21 days of exposure, daphnid survival and reproduction were evaluated. There was no statistically significant chemical effects at any exposure level, however, the reproduction data for the highest treatment level suggested the slight possibility of an effect. Therefore, it was decided to extend the study an additional 7 days in an effort to establish if a significant effect could be confirmed at the highest exposure concentration.

A summary of the survival data from the chronic exposure of *Daphnia magna* to crotonaldehyde is presented in Table 4 and illustrated in Figure 3. At the termination of the 28-day study, survival rates of daphnids exposed to the 1.5, 0.76, 0.38, 0.19 and 0.095 mg A.I./L treatment levels were 100, 95, 100, 85 and 100%, respectively, and was not

statistically different from the performance of daphnids exposed to the control solutions (98%). The 28-day EC50 for this study was >1.5 mg A.I./L crotonaldehyde. At test termination, no adverse sublethal effects were observed among daphnids at any treatment levels. Observations of parental daphnids exhibiting abnormal behavior or appearance is presented in Table 5.

A summary of the cumulative number of offspring produced by daphnids and observations of young exposed to the concentrations of crotonaldehyde is presented in Table 6 and Table 7. Control daphnids began releasing offspring by test day 8. By test termination, control daphnids had produced an average of 345 offspring. The time to first offspring release and the total number of offspring produced by control organisms greatly exceeded criteria by U.S. EPA (1985). Release of first brood offspring by daphnids exposed to treatment level solutions ≤1.5 mg A.I./L occurred by test day 8 and was not adversely affected by crotonaldehyde. At test termination, the mean reproduction of daphnids exposed to the 1.5, 0.76, 0.38, 0.19 and 0.095 mg A.I./L treatment levels averaged 317, 329, 315, 292 and 323 offspring/female, respectively. Statistical analysis (Dunnett's procedure) established that the reproductive performance of organisms (317 offspring/female) in the highest treatment level (1.5 mg A.I./L) was not statistically different from (345 offspring/female) the organisms in the control solutions. Based on the absence of a statistically determined adverse effect in daphnid reproduction or survival at the highest treatment level tested, it was established that crotonaldehyde concentrations of ≤1.5 mg A.I./L were not chronically toxic to *D. magna*. Copies of raw data used to establish the maintained exposure conditions (e.g., water quality, test article concentration analyses) and the concentration-effect response used to determine the reported NOEC and MATC for this study are presented in Appendix 8.

8.0 TEST VALIDITY

The following criteria for a valid test were met during the study:

A. Immobilization, stress or disease among control daphnids did not exceed 20%.

B. Control daphnids reproduced at a rate of ≥ 60 offspring/female in 21 days.

C. No ephippia were produced by control organisms.

9.0 PROTOCOL DEVIATIONS

No deviations to the protocol were noted.

10.0 CONCLUSION

A preliminary test was conducted prior to the initiation of the definitive test. Following 11 days of exposure, immobilization ranging from 0 - 35% was observed in the treatment levels (750 - 47 µg A.I./L, nominal). During the same period, reproduction among daphnids exposed to the highest nominal test concentration, 750 µg A.I./L was 19 offspring/female. Reproduction in the remaining nominal test concentrations (380 - 47 µg A.I./L) ranged from 39 - 48 offspring/female. Reproduction among control daphnids averaged 40 offspring/female.

A definitive study was conducted for 21 days, whereupon survival and reproduction were evaluated. There were no statistically significant chemical effects at any exposure level, however, the reproduction data for the highest treatment level suggested the slight possibility of an effect. Based upon the absence of an adverse effect on daphnid survival or reproduction, the NOEC was defined as 1.5 mg A.I./L, the highest nominal concentration tested during the 28-day chronic exposure.

An acute flow-through study was performed by Eastman Kodak Company (Study No. EN-407-901878-1), exposing daphnids to nominal treatment levels of 10, 5, 2.5, 1.2 and 0.6 mg A.I./L crotonaldehyde. Based on data obtained during this study, the 48-hour EC50 (95% confidence intervals) was calculated to be 2.0 (1.3 - 2.5) mg/L. The Lowest-Observed-Effect Concentration was statistically determined to be 2.5 mg A.I./L.

Based on the results of the acute and chronic studies conducted at Eastman Kodak Company and Springborn Laboratories, it was established that acute exposure to concentrations ≥2.5 mg A.I./L adversely affect the survival of *D. magna*. Chronic exposure to levels less than those concentrations which elicited an adverse response during an acute exposure, did not adversely affect the organisms' survival or reproductive performance. Therefore, utilizing the Lowest-Observed-Effect Concentration (LOEC) determined during the acute study (i.e., 2.5 mg A.I./L) and No-Observed-Effect Concentration (NOEC) determined during the chronic exposure (i.e., 1.5 mg A.I./L), the MATC for crotonaldehyde and *D. magna* was estimated as >1.5 mg A.I./L and <2.5 mg A.I./L (Geometric Mean MATC = 1.9 mg A.I./L). The Acute/Chronic Ratio (ACR) for crotonaldehyde and *D. magna* (i.e., 48-hour EC50, divided by the estimated Geometric Mean MATC; 2.0/1.9), was calculated to be 1.

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TABLES

Table 1. Stability of crotonaldehyde in water.

Matrix	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL) 0-Hour	24-Hour	48-Hour[a]	96-Hour
Hard water[b]	17	2.329	3.134	---[c]	NS
		2.384	1.356	---[c]	
Soft water[d]	17	12.510	1.709	1.650	NS
		13.070	---[c]	---[c]	
NANOpure® water	17	14.790	14.200	---[c]	19.600
		14.730	15.350	---[c]	16.540
	QC #1[e]	16.940	18.52	16.62	19.140
	QC #2	17.33	10.18[f]	16.79	21.910
	QC #3	25.16[f]	11.89[f]	25.180[f]	22.110

[a] Analytical difficulties were experienced during the preparation and the analysis of the experimental samples removed at 48 hours.
[b] Hardness equal to approximately 170 mg/L as $CaCO_3$.
[c] Below the limit of quantitation
[d] Hardness equal to approximately 26 mg/L as $CaCO_3$.
[e] Quality Control samples prepared in NANOpure® water, nominal = 20.2 mg/L
[f] Percent recovery for this QC sample was outside of the standard range accepted by this laboratory (i.e., ± 3 standard deviations from the mean recovery established during the method validation/recovery study, Appendix 5).

NS = Not Sampled

Table 2. **Results of the measurement of water quality parameters in the test solutions during the 28-day chronic exposure of daphnids (*Daphnia magna*) to crotonaldehyde.**

Mean (Standard Deviation)

Nominal Concentration (mg A.I./L)	Dissolved[a] Oxygen (mg/L)	Temperature[b] (°C)	Total[c] Hardness (mg/L $CaCO_3$)	Total[c] Alkalinity (mg/L $CaCO_3$)	Specific[c] Conductance (μmhos/cm)	pH[a] Range
1.5	8.0(0.5)	20(0.7)	170(5)	110(2)	500(0)	8.1-8.3
0.76	8.2(0.5)	20(0.7)	---[d]	---[d]	500(0)	8.1-8.3
0.38	8.3(0.5)	20(0.7)	---[d]	---[d]	500(0)	8.0-8.3
0.19	8.4(0.4)	20(0.7)	---[d]	---[d]	500(0)	8.0-8.3
0.095	8.5(0.4)	20(0.7)	170(2)	110(1)	500(0)	8.0-8.3
Control	8.5(0.4)	20(0.7)	170(6)	110(2)	500(0)	8.0-8.3

[a] N = 44, based on measurements of each replicate exposure vessel (of all test concentrations and the control) at test initiation and weekly thereafter. On the remaining test days, measurements were performed in one replicate vessel of all test concentrations and the control.

[b] N = 44 (based on daily and weekly measurement of each treatment level and control, Brooklyn alcohol thermometer)

[c] N = 5 (based on measurement of one replicate exposure vessel at test initiation and weekly thereafter)

[d] Measurement not required at this concentration level.

Table 3. Results of the analysis of stock solutions for crotonaldehyde during the 28-day chronic exposure of *Daphnia magna*.

Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)				
	Day 0	Day 7[a]	Day 14	Day 21	Day 28[b]
17	23.26	20.55	22.85	20.04	13.74[c]
	21.28	20.45	21.41	18.67	14.28
	7.399[d]	20.79	22.45	20.20	
QC[e]#1	16.4	24.4[g]	18.6	21.5	18.1
	(20.2)[f]	(20.2)	(20.2)	(20.2)	(20.2)
QC#2	19.2	24.5[g]	18.5	22.2	16.7
	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)
QC#3	20.3	24.3[g]	18.9	22.0	18.5
	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)

[a] Stock solution was 1 day old at the time of sampling.
[b] Stock solution was 7 days old at the time of sampling.
[c] Two samples were removed and analyzed for the purpose of confirming concentration in the stock solution prepared and placed on the diluter system on day 21 of exposure.
[d] Value recognized as below expected concentration due to analytical difficulties and is not representative of the stock solution.
[e] QC = Quality Control sample
[f] Nominal fortified concentration is presented in parentheses.
[g] Percent recovery is outside the standard acceptable range established by this laboratory (i.e. ± 3 standard deviations from the mean recovery established during the method validation, Appendix 5).

Table 4. **Mean percent survival of parental daphnids (*Daphnia magna*) during the 28-day chronic exposure to crotonaldehyde.**

Nominal Concentration (mg A.I./L)		Mean Percent Survival												
	Day:	1	2	4	7	9	11	14	16	18	21	23	25	28
1.5	A	100	100	100	100	100	100	100	100	100	100	100	100	100
	B	100	100	100	100	100	100	100	100	100	100	100	100	100
	C	100	100	100	100	100	100	100	100	100	100	100	100	100
	D	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100
0.76	A	100	100	100	100	100	100	100	100	100	100	100	100	100
	B	100	100	100	100	100	100	100	100	100	100	90	90	90
	C	100	100	100	100	100	100	100	100	100	100	100	100	90
	D	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	98	98	95
0.38	A	100	100	100	100	100	100	100	100	100	100	100	100	
	B	100	100	100	100	100	100	100	100	100	100	100	100	100
	C	100	100	100	100	100	100	100	100	100	100	100	100	100
	D	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100
0.19	A	100	100	100	100	100	100	100	100	100	100	100	100	
	B	100	100	100	100	100	100	100	100	100	100	90	90	90
	C	100	100	100	100	100	100	90	90	90	90	90	80	80
	D	100	100	100	100	100	100	70	70	70	70	70	70	70
	Mean	100	100	100	100	100	100	90	90	90	90	88	85	85
0.095	A	100	100	100	100	100	100	100	100	100	100	100	100	100
	B	100	100	100	100	100	100	100	100	100	100	100	100	100
	C	100	100	100	100	100	100	100	100	100	100	100	100	100
	D	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	100	100	100	100
Control	A	100	100	100	100	100	100	100	100	100	100	100	100	100
	B	100	100	100	100	100	100	100	100	100	90	90	90	90
	C	100	100	100	100	100	100	100	100	100	100	100	100	100
	D	100	100	100	100	100	100	100	100	100	100	100	100	100
	Mean	100	100	100	100	100	100	100	100	100	98	98	98	98

Table 5. **Mean percent of parental daphnids (*Daphnia magna*) exhibiting abnormal behavior or appearance during the 28-day chronic exposure to crotonaldehyde.**

Nominal Concentration (mg A.I./L)	DAY												
	1	2	4	7	9	11	14	16	18	21	23	25	28
1.5	None	None	None	None	None	None	None	None	None	None	None	None	None
0.76	None	None	None	None	None	None	None	None	None	None	None	None	None
0.38	None	None	None	None	None	None	None	None	None	None	None	None	None
0.19	None	None	None	None	None	5% A	None	3% B	3% B	6% B	None	None	None
0.095	None	None	None	None	None	None	None	None	None	None	None	None	None
Control	None	None	None	None	None	None	None	None	None	None	None	None	None

[a] Daphnids were observed to be pale and on the bottom of the test vessel
[b] Daphnids were observed to be pale

Table 6. **Cumulative number of offspring produced per female *Daphnia magna* during the 28-day chronic exposure to crotonaldehyde.**

Nominal Concentration (mg A.I./L)		Mean Cumulative Number of Offspring/Female									
	Day:	7	9	11	14	16	18	21	23	25	28
1.5	A	0	12	54	99	99	144	196	204	260	319
	B	0	10	47	95	95	146	211	235	269	329
	C	0	10	43	83	83	128	186	193	249	304
	D	0	9	46	93	97	139	196	226	258	314
	Mean	0	10	48	93	94	139	197	215	259	317
0.76	A	0	11	58	115	115	171	230	259	289	340
	B	0	12	55	108	114	164	222	243	282	341
	C	0	9	51	99	104	145	199	224	272	333
	D	0	7	38	74	83	125	179	200	241	300
	Mean	0	10	51	99	104	151	208	232	271	329
0.38	A	0	9	42	91	91	142	202	234	258	307
	B	0	12	50	98	98	164	224	245	286	339
	C	0	11	42	90	93	145	201	211	251	305
	D	0	10	45	95	95	148	205	220	262	308
	Mean	0	11	45	94	94	150	208	228	264	315[a]
0.19	A	0	7	44	96	96	145	190	206	242	295
	B	0	12	54	111	111	144	188	206	242	304
	C	0	13	47	88	88	140	199	200	258	313
	D	0	8	46	77	77	105	144	174	193	255
	Mean	0	10	48	93	93	134	180	197	234	292[a]
0.095	A	0	21	51	97	97	149	207	220	263	308
	B	0	8	52	110	110	165	231	244	296	340
	C	0	11	54	97	97	152	209	229	271	317
	D	0	11	51	97	97	153	214	243	273	327
	Mean	0	13	52	100	100	155	215	234	276	323
Control	A	0	7	50	106	106	171	241	268	307	349
	B	0	10	42	92	92	150	212	212	280	341
	C	0	12	57	107	107	167	241	259	308	359
	D	0	10	47	94	94	148	217	218	280	330
	Mean	0	10	49	100	100	159	228	239	294	345

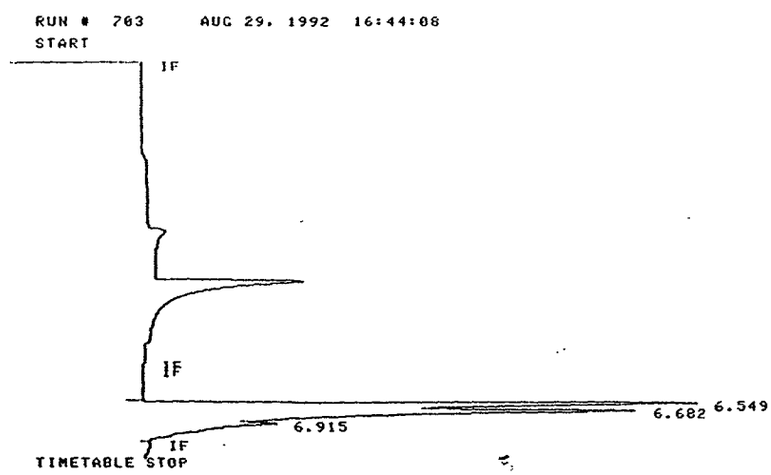
[a] Significantly reduced ($p \leq 0.05$) when compared to the control. However, in view of the lack of statistical differences at higher test concentrations, is not considered biologically significant.

Table 7. **Mean percent of offspring (*Daphnia magna*) exhibiting abnormal behavior or appearance during the 28-day chronic exposure to crotonaldehyde.**

Nominal Concentration (mg A.I./L)	Day: 7	9	11	14	16	18	21	23	25	28
1.5	None	None	None	None	None	None	None	None	None	None
0.76	None	None	None	None	None	None	None	None	None	None
0.38	None	None	None	None	None	None	None	None	None	None
0.19	None	None	None	None	None	None	None	None	None	None
0.095	None	None	None	None	None	None	None	None	None	None
Control	None	None	None	None	None	None	None	None	None	None

FIGURES

Figure 1. Representative chromatogram showing recovery of crotonaldehyde from the stock solution.



RUN# 703 AUG 29, 1992 16:44:08

SAMPLE NAME: 8-92-1234 SAMPLE# 30
17000 MG/L

IDENTIFIER : 2728A12358

CROT

ESTD%-AREA

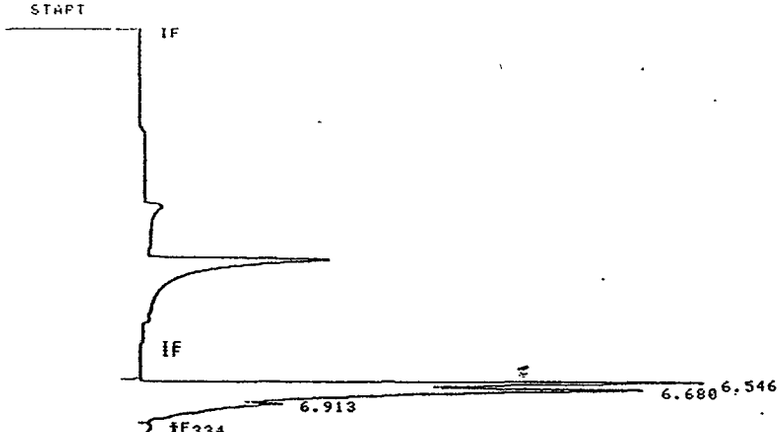
RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	MG/L	NAME
6.600	++	42582816	.114	6199866	1R	2363.798	CROT

TOTAL AREA=4.2583E+07
MUL FACTOR=4.0000E+03
SAMPLE AMT=4.0000E+03

Figure 2. **Representative chromatogram showing recovery of crotonaldehyde from one of the Quality Control samples analyzed concurrently with the stock solution.**

```
TOTAL AREA=3.7397E+07
MUL FACTOR=4.0000E+03
SAMPLE AMT=4.0000E+03

RUN #  702      AUG 29, 1992  16:33:50
STRT
```




```
TIMETABLE STOP

RUN#  .702.          AUG 29, 1992  16:33:50

SAMPLE NAME: 8-92-1233 QA      SAMPLE#   29
20000 MG/L
20200
IDENTIFIER : 2728A12358

CROT

EST0%-AREA
      RT TYPE       AREA  WIDTH     HEIGHT CAL# MG/L        NAME
   6.600   ++   42644192   .116    6109328   LR   2367.205  CROT
   7.334 I VH     587976   .195      50275              .000

TOTAL AREA=4.3232E+07
MUL FACTOR=4.0000E+03
SAMPLE AMT=4.0000E+03
```

Figure 3. **Mean percent survival of daphnids (*Daphnia magna*) during the 28-day chronic exposure to crotonaldehyde.**

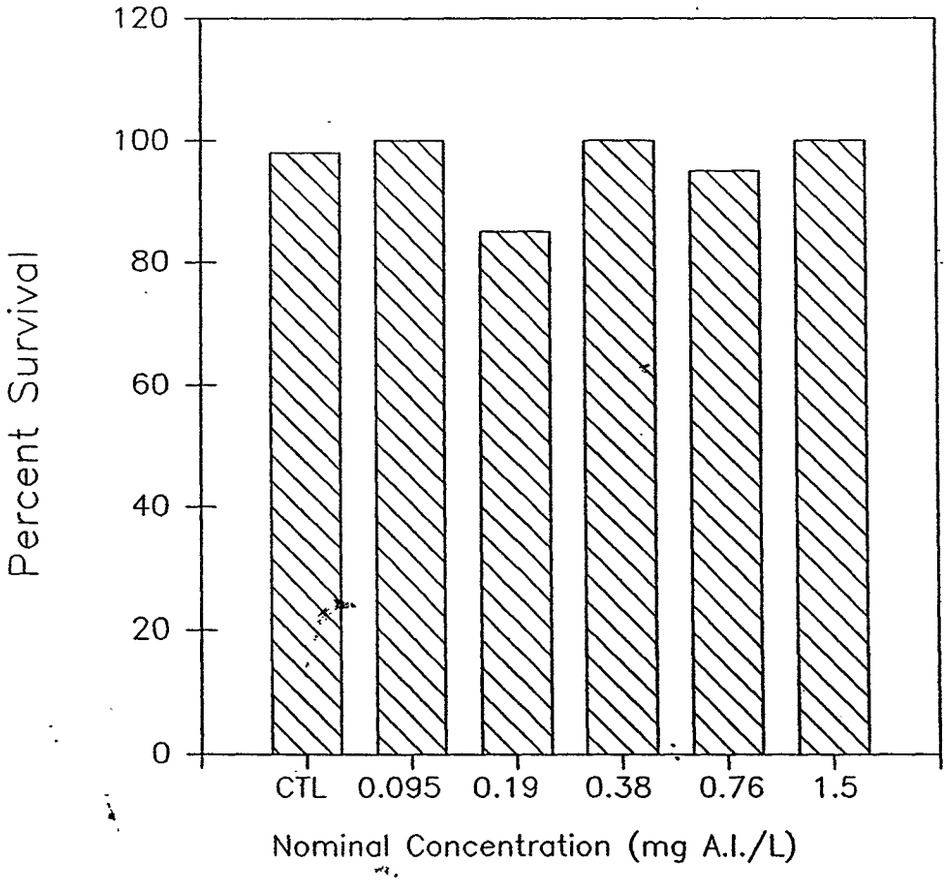
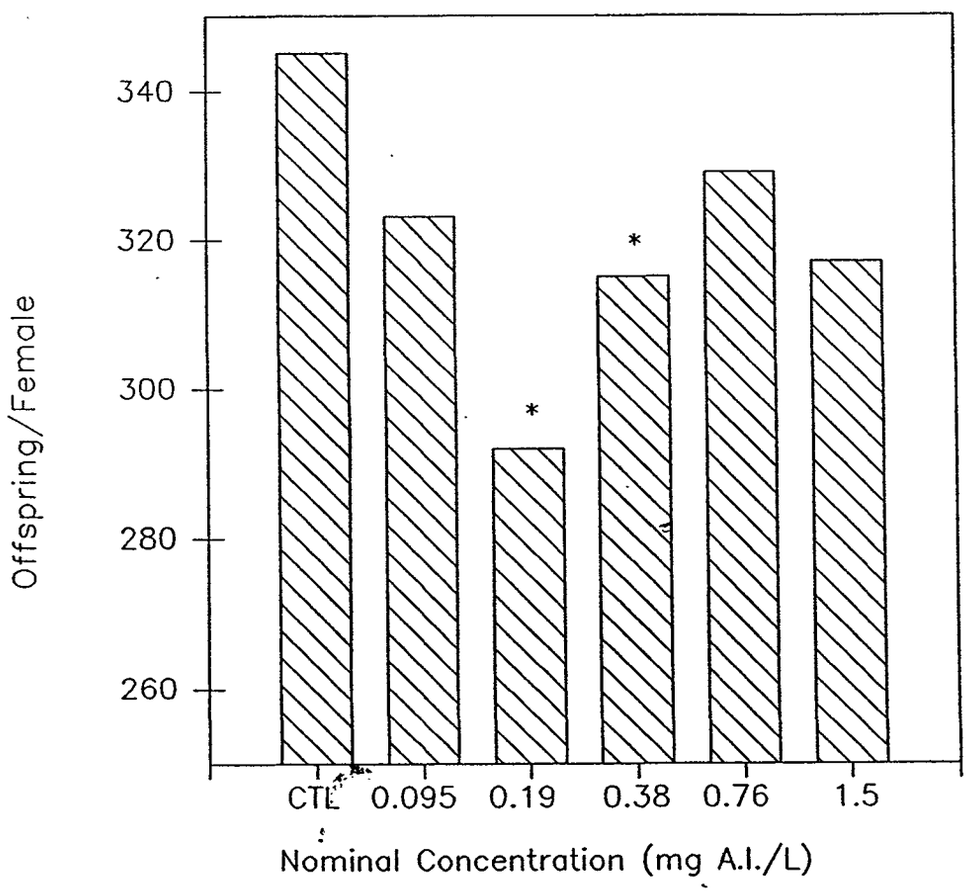


Figure 4. **Cumulative number of offspring/female *Daphnia magna* exposed to crotonaldehyde during the 28-day chronic toxicity study.**



* Significantly reduced ($p \leq 0.05$) when compared to the control. However, in view of the lack of statistical differences at higher test concentrations, this effect is not considered biologically significant.

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APPENDIX 1 - MATERIAL SAFETY DATA SHEET

901878

MATERIAL SAFETY DATA SHEET

EASTMAN CHEMICAL PRODUCTS, INC.
EASTMAN KODAK COMPANY
Kingsport, Tennessee 37662

For Health Hazard Information, Call: (615) 229-6094

For Other Information, Call Your Eastman Representative

Eastman Operator: (615) 229-2000 Date of Preparation 08-24-87

SECTION I. IDENTIFICATION

-- Name:

Crotonaldehyde

-- Synonyms: PM 161; 2-Butenal,
-- Formula: C_4H_6O
-- Molecular Weight: 70.09

SECTION II. PRODUCT AND COMPONENT HAZARD DATA

A. COMPONENTS:	Approx Weight %	CAS Reg No	Eastman Kodak No
Crotonaldehyde*	92	4170-30-3	901878
Water	8		

See Section VI-A for information on exposure limits.

B. PRECAUTIONARY LABEL STATEMENTS:

DANGER! FLAMMABLE
MAY BE FATAL IF INHALED OR ABSORBED THROUGH THE SKIN
CAUSES SKIN AND EYE BURNS
HARMFUL IF SWALLOWED
VAPOR EXTREMELY IRRITATING
MAY FORM EXPLOSIVE PEROXIDES
MAY POLYMERIZE

Keep away from heat, sparks, and flame.
Do not breathe vapor.
Do not get in eyes, on skin, on clothing.
Keep container tightly closed.
Use only with adequate ventilation.
Wash thoroughly after handling.
Do not allow to evaporate to near dryness.
Keep from contact with alkaline materials.

POISON-INHALATION HAZARD CALL A PHYSICIAN IMMEDIATELY

MSDS-10,597A-1 (08-87)
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FIRST AID: If inhaled, remove to fresh air. If not breathing, give artificial respiration, preferably mouth to mouth. If breathing is difficult, give oxygen. In case of contact, immediately flush eyes and skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Destroy contaminated shoes. If swallowed, DO NOT INDUCE VOMITING. If conscious, give one glass of milk or water. Never give anything by mouth to an unconscious person.

IN CASE OF FIRE: Use water spray, dry chemical, "alcohol" foam, or CO_2. Water may be ineffective in fighting the fire. Use water spray to keep fire-exposed containers cool.

IN CASE OF SPILL: Emergency personnel should wear self-contained breathing apparatus. Eliminate all ignition sources. Use water spray to disperse vapors and to flush spill area. Prevent runoff from entering drains, sewers, and streams.

Since emptied containers retain product residue, follow label warnings even after container is emptied. Do not cut, drill, grind, or weld on or near
this container.

FOR MANUFACTURING USE ONLY

SECTION III. PHYSICAL DATA (1)

-- Appearance and Odor: Clear, colorless liquid; pungent, suffocating odor; lachrymator.
-- Boiling Point: 84°C (183°F).
-- Specific Gravity (H_2O = 1): 0.871.
-- Vapor Pressure: 32 mm Hg at 20°C.
-- Percent Volatile by Volume: Approx 1.0.
-- Vapor Density (Air = 1): 2.41.
-- Evaporation Rate (ethyl ether = 1): 0.2.
-- Solubility in Water: Appreciable.

SECTION IV. FIRE AND EXPLOSION HAZARD DATA (1)

-- Flash Point: 7°C (45°F); Method Used: Tag Closed Cup.
-- Autoignition Temperature: 160°C (320°F); Method Used: ASTM E 659.
-- Cool Flame Autoignition Temperature: 121°C (250°F).
-- Flammable Limits: LEL 2.15% at 75°F.
UEL 19.5% at 165°F.
-- Extinguishing Agent: Water spray, dry chemical, CO_2, or "alcohol" foam.
-- Special Fire-Fighting Procedures: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Water may be ineffective for fire fighting. Use water spray to keep fire-exposed containers cool.
-- Unusual Fire and Explosion Hazards: Flammable liquid (see Section VIII). At elevated temperatures, such as in fire conditions, polymerization may take place. If the polymerization takes place in a container, there is a possibility of violent rupture of the container. Vapors are heavier than air and may travel along the ground or may be moved by ventilation to an ignition source and may flash back.

MSDS-10,597A-2 (08-87)
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SECTION V. REACTIVITY DATA (1)

-- Stability: Stable at ambient temperatures; however, may polymerize at elevated temperatures. The material readily oxidizes to an acid and may form explosive peroxides on exposure to air.
-- Stability calculated by ASTM CHETAH 4.3: Sensitive.
Heat of decomposition: -0.71 kcal/g.
Heat of combustion: -7.48 kcal/g.
-- Incompatibility: Oxidizing and alkaline materials can cause a vigorous reaction. Also see "Hazardous Polymerization" below.
-- Hazardous Decomposition Products: As with any other organic material, combustion will produce carbon dioxide and probably carbon monoxide.
-- Hazardous Polymerization: May occur. Conditions to Avoid: Violent polymerization may occur upon contact with alkaline materials such as caustic, ammonia or amines. Polymerization will also occur at elevated temperatures.

SECTION VI. TOXICITY AND HEALTH

A. EXPOSURE LIMITS

-- OSHA Permissible Exposure Limit (PEL): 2 ppm-TWA.
-- Threshold Limit Value (TLV): 2 ppm-TWA, ACGIH, 1986-87.
-- A NIOSH industrial hygiene analytical method is available. (2)

B. EXPOSURE EFFECTS

Ingestion: Harmful if swallowed.

Inhalation: May be fatal if inhaled. Vapor causes severe upper respiratory tract irritation.

Eyes: Liquid causes severe burns. Vapor extremely irritating.

Skin: May be fatal if absorbed through the skin. Causes burns.

C. FIRST AID

Ingestion: DO NOT INDUCE VOMITING. If conscious, give one glass of milk or water. Never give anything by mouth to an unconscious person. Call a physician immediately.

Inhalation: Remove to fresh air. If not breathing, give artificial respiration, preferably mouth to mouth. If breathing is difficult, give oxygen. Call a physician immediately.

Eyes: Immediately flush with plenty of water for at least 15 min. Call a physician.

Skin: Immediately flush with plenty of water for at least 15 min while removing contaminated clothing and shoes. Call a physician immediately. Wash contaminated clothing before reuse. Destroy contaminated shoes.

MSDS-10,597A-3 (08-87)
Replaces 07-87 Edition

D. TOXICITY DATA

Test	Species	Result	Toxicity Classification (3)
Acute oral LD_{50}	Rat	300 mg/kg (4)	Moderately toxic
Dermal LD_{50}	Rabbit	150 to 200 mg/kg (4)	Moderately toxic
Dermal LD_{50}	Rabbit	380 mg/kg (4)	Slightly toxic
Dermal LD_{50}	Guinea pig	500 to 1000 mg/kg (4)	
Inhalation LC_{50}	Rat	600 ppm/0.5 h (5)	
Inhalation LC_{50}	Rat	380 ppm/1 h (5)	
Inhalation LC_{50}	Rat	85 ppm/4 h (5)	Highly toxic
Eye irritation	Rabbit	Severe (4)	

SECTION VII. VENTILATION AND PERSONAL PROTECTION

A. VENTILATION:

Good general ventilation (typically 10 air changes per hour) should be used. Ventilation rates should be matched to conditions. Normally, local exhaust ventilation or an enclosed handling system will be needed to control airborne levels below recommended exposure limits (see Section VI-A).

B. RESPIRATORY PROTECTION:

An appropriate full-face NIOSH-approved respirator for organic vapor must be worn if exposure is likely to exceed recommended exposure limits (see Section VI-A). If respirators are used, a program should be established to assure compliance with OSHA Standard 29 CFR 1910.134.

C. SKIN AND EYE PROTECTION:

Wear safety glasses with side shields (or goggles) and a face shield. Impermeable gloves should be worn. An impermeable apron or smock and boots should be worn to minimize skin contact. A safety shower, an eye bath, and washing facilities should be available. Wash thoroughly after handling.

SECTION VIII. SPECIAL STORAGE AND HANDLING PRECAUTIONS

Material is classified as a Flammable Liquid. Keep away from heat, sparks, and flame. Keep container closed. Use with adequate ventilation. Vapors are heavier than air and may travel along the ground or may be moved by ventilation to an ignition source and flash back. Possible peroxide former. Do not evaporate to near dryness. Keep container tightly closed. Do not contaminate. Since emptied containers retain product residue, follow label warnings even after container is emptied. Do not cut, drill, grind, or weld on or near this container.

SECTION IX. SPILL, LEAK, AND DISPOSAL PRACTICES

Steps to be Taken in Case Material is Released or Spilled: Wear appropriate protective clothing (including a self-contained breathing apparatus). Eliminate all ignition sources. Small spills may be collected with absorbent materials. For large spills, use water spray to disperse vapors and to flush area. Prevent runoff from entering drains, sewers, or streams. Clean Water Act and Superfund reportable quantity (RQ): 111 Lbs.

MSDS-10,597A-4 (08-87)
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Waste Disposal Method: Mix with compatible chemical which is less flammable and incinerate. Observe all federal, state, and local laws concerning health and environment.

SECTION X. ENVIRONMENTAL EFFECTS DATA

A. SUMMARY

Some laboratory data and published data are available for this product, and these data (6-8) have been used to provide the following estimate of environmental impact:

This product has a moderate to high biological oxygen demand, and it may cause oxygen depletion in aquatic systems. It has a high potential to affect aquatic organisms. This product is biodegradable and is not expected to persist in the environment. The direct, instantaneous discharge to a receiving body of water of an amount of this product which will rapidly produce by dilution a final concentration of 0.13 mg/L or less is not expected to have any adverse environmental impact. After dilution with a large amount of water, followed by secondary waste treatment, this product is not expected to have any adverse environmental impact.

B. OXYGEN DEMAND DATA

-- ThOD: 2.28 g/g (6)
-- COD: 97% of ThOD (7)
-- BOD_5: 1.54 g/g (6); 37% of ThOD (7)
-- BOD_{10}: 1.30 g/g (7)

C. ACUTE AQUATIC EFFECTS

-- 96-h LC_{50}; Bluegill sunfish: 3.5 mg/L (7,8)
-- 96-h LC_{50}; Tidewater silversides: 1.3 mg/L (7,8)

SECTION XI. TRANSPORTATION

DOT Hazard Classification: Flammable liquid (Poison - Inhalation hazard).
Flashpoint: See Section IV.
Proper DOT Shipping Name: Crotonaldehyde.
UN Number: 1143.

SECTION XII. REFERENCES

1. File data, Material Safety Program, Eastman Chemicals Division, Eastman Kodak Company, Kingsport, Tennessee.

2. NIOSH Manual of Analytical Methods, 2nd Edition, Volume 5. Issued by the National Institute for Occupational Safety and Health. Washington, U. S. Government Printing Office, 1979, Method 285.

3. AM IND HYG ASSOC Q 10, 93-96 (1949).

4. G. D. Clayton and F. E. Clayton, Editors. PATTY'S INDUSTRIAL HYGIENE AND TOXICOLOGY, 3rd Revised Edition, Volume 2A. New York, Wiley-Interscience, 1981, p. 2651.

5. AM IND HYG ASSOC J 28, 561-566 (1967).

MSDS-10,597A-S (08-87)
Replaces 07-87 Edition

6. Unpublished data, Health and Environment Laboratories, Eastman Kodak Co., Rochester, New York.

7. K. Verschueren. HANDBOOK OF ENVIRONMENTAL DATA ON ORGANIC CHEMICALS, 2nd Edition. Van Nostrand Reinhold Company, New York, 1983, pp. 410-411.

8. J HAZARDOUS MATER 1, 303-318 (1977).

SECTION XIII. HAZARD RATINGS

	Health	Flammability	Reactivity
HMIS* Rating:	3	3	2
NFPA** Rating:	3	3	2

NOTICE: These ratings involve data and interpretations that may vary from company to company and are intended only for rapid, general identification of the magnitude of the specific hazard. TO DEAL ADEQUATELY WITH THE SAFE HANDLING OF THIS MATERIAL, ALL THE INFORMATION CONTAINED IN THIS MSDS MUST BE CONSIDERED. The customer is responsible for determining the proper personal protective equipment needed for its particular use of this material.

*Hazardous Materials Identification System's [HMIS] Revised RAW MATERIALS RATING MANUAL, National Paint & Coatings Association, Fall 1984.

**NFPA 704 Standard System for the Identification of the Fire Hazards of Materials, National Fire Protection Association, 1985.

The information contained herein is furnished without warranty of any kind. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

TX1166S/901878/R-3, S-3, F-3, C-2

MSDS-10,597A-6 (08-87)
Replaces 07-87 Edition

APPENDIX 2 - PURITY DETERMINATION

ANALYTICAL TEST REPORT

Crotonaldehyde

Accession Number: 901878 HAEL Number: 92-0072

BY

Beth Isaacs

TESTING FACILITY

Environmental Analytical Services
Chemicals Quality Services Division
Eastman Kodak Company
1100 Ridgeway Avenue
B-320 Kodak Park
Rochester, New York 14652-3615

SPONSOR

Eastman Kodak Company
B-320 Kodak Park
Rochester, New York 14652-3615

Completion Date: 09/09/92

Accession No.: 901878 HAEL No.: 92-0072

STUDY TYPE

Environmental Studies

REQUESTED BY

Kenneth A. Robillard Ph.D.

REQUEST #: 141591

TEST SUBSTANCE

Name: Crotonaldehyde
Accession No.: 901878
HAEL No.: 92-0072
Lot No.: 7-92

DATES OF EXPERIMENT

Date received: 07/21/92
Date analyzed: 08/13/92
Date reported: 08/14/92

ANALYTICAL PERSONNEL

Beth Isaacs, Laboratory Technician

ANALYTICAL DIRECTOR

Barry W. Remington

DATA STORAGE AND RECORD RETENTION

All original raw data will be archived for at least ten years by the Chemicals Quality Sevices Division B-320 of the Eastman Kodak Co., Kodak Park, Rochester, New York 14652.

Accession No.: 901878 HAEL No.: 92-0072

METHODS:

One sample was received for a purity determination. The sample was analyzed by gas chromatography (GC) using the following instrument conditions:

Instrument:	Hewlett Packard 5890
Column:	J&W; DB Wax; 30M; wide bore; 0.25um film thickness
Carrier Gas:	Helium
Column Pressure:	7 psig
Split Flow:	120 cc/min.

Temperature Program:

Initial Temp.:	50°C
Initial Hold Time:	4 min.
Rate:	10°C/min.
Final Temp.:	250°C
Final Hold Time:	7 min.

Injection Port:	250°C
Injection Type:	split
Injection Volume:	1 uL
Detector:	Flame Ionization Detector (FID)
Detector Temp.:	250°C
Diluting Solvent:	2-Propanol

Accession No.: 901878 HAEL No.: 92-0072

RESULTS

The test chemical was diluted with 2-propanol to determine the purity. This solution was then analyzed on 08/14/92 by GC/FID. The following results are the average of three injections:

mean = 99.9% 92-0072
std. dev. = 0.0000
n = 3

ANALYST Beth Isaacs DATE 8.14.92
Beth Isaacs

REVIEWED BY [signature] DATE 9/9/92

Accession No.: 901878 HAEL No.: 92-0072

ANALYTICAL QUALITY ASSURANCE INSPECTION STATEMENT
(CFR 58.35(B)(7) 792.35(B)(7) 160.35(B)(7))

STUDY: 92-0072-Z STUDY DIRECTOR:
ANALYTICAL DIRECTOR: REMINGTON, B.
KAN : 901878
CQS JOB NUMBER: 3Z13N

STUDY TYPE: ANALYTICAL TESTING FOR ENVIRONMENTAL STUDIES

(AUDITOR, QUALITY ASSURANCE UNIT) 5/5/92 DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF HQAO, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECT DATES	REQUEST NUMBER	PHASE(S) INSPECTED	STATUS REPORT DATES
09/09/92	141591	PURITY TEST REPORT INSPECTION	09/09/92

ANALYTICAL TEST REPORT

Crotonaldehyde

KAN: 901878 HAEL Number: 92-0072

BY

Beth Isaacs

TESTING FACILITY

Environmental Analytical Services
Chemicals Quality Services Division
Eastman Kodak Company
1100 Ridgeway Avenue
B-320 Kodak Park
Rochester, New York 14652-3615

SPONSOR

Eastman Kodak Company
B-320 Kodak Park
Rochester, New York 14652-3615

Completion Date: 10/07/92

Page 1 of 6

KAN: 901878 HAEL No.: 92-0072

STUDY TYPE

Environmental Studies

REQUESTED BY

Kenneth A. Robillard Ph.D.

REQUEST #: 141591

TEST SUBSTANCE

Name: Crotonaldehyde
Accession No.: 901878
HAEL No.: 92-0072
Lot No.: 7-92

DATES OF EXPERIMENT

Date received: 07/21/92
Date analyzed: 09/17/92
Date reported: 09/23/92

ANALYTICAL PERSONNEL

Beth Isaacs, Laboratory Technician

ANALYTICAL DIRECTOR

Barry W. Remington

DATA STORAGE AND RECORD RETENTION

All original raw data will be transferred to the Environmental Sciences Section of the Corporate Health and Environment Laboratories of the Eastman Kodak Co., Kodak Park, Rochester, New York 14652-3617.

KAN: 901878 HAEL No.: 92-0072

METHOD:

One sample was received for a percent moisture determination. The sample was analyzed by gas chromatography (GC) with a thermal conductivity detector (TCD), using the following instrument conditions:

Instrument:	Hewlett Packard 5890
Column:	Chrompack; plot fused silica; 25m x 0.32mm; coating poraplot Q
Carrier Gas:	Helium
Column Pressure:	12 psig
Col. + Aux. Flow:	4.0 mL/min.
Reference Flow:	15 mL/min.
Split Flow:	65 mL/min.

Temperature Program:

Initial Temp.:	80°C
Initial Hold Time:	0 min.
Rate:	10°C/min.
Final Temp.:	200°C
Final Hold Time:	5 min.

Injection Port:	200°C
Injection Type:	split
Injection Volume:	1 uL
Detector:	Thermal Conductivity Detector (TCD)
Detector Temp.:	240°C
Diluting Solvent:	2-Propanol (for standards only)

KAN: 901878 HAEL No.: 92-0072

RESULTS

The test chemical was analyzed neat on 09/17/92 by GC/TCD to determine the percent moisture. The following results are the average of two injections:

Injection	% H_2O (v/v)
1	6.2
2	6.0

mean = 6.1% (v/v)
n = 2

KAN: 901878 HAEL No.: 92-0072

Signature Page:

ANALYST Beth Isaacs DATE 9.23.92
Beth Isaacs

REVIEWED BY [signature] DATE 10-7-92

KAN: 901878 HAEL No.: 92-0072

QUALITY ASSURANCE STATEMENT

ANALYTICAL QUALITY ASSURANCE INSPECTION STATEMENT
(CFR 58.35(B)(7) 792.35(B)(7) 160.35(B)(7))

STUDY: 92-0072-Z STUDY DIRECTOR:
ANALYTICAL DIRECTOR: REMINGTON, B.
KAN : 901878
CQS JOB NUMBER: 3Z13N

STUDY TYPE: ANALYTICAL TESTING FOR ENVIRONMENTAL STUDIES

(AUDITOR, QUALITY ASSURANCE UNIT) 10/6/92 DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF MQAO, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECT DATES	REQUEST NUMBER	PHASE(S) INSPECTED	STATUS REPORT DATES
10/06/92	141591	MOISTURE DETERMINATION TEST REPORT INSPECTION	10/06/92

APPENDIX 3 - STUDY PROTOCOL

RE

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with *Daphnia magna* Following TSCA Test Standard No. 797-1330.

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: Eastman Kodak Company

Address: Environmental Sciences Section, Corporate Health and Environment Laboratories
Rochester, NY 14652-3617 **Phone:** 716/588-2140

Sponsor Protocol/Project No.:

Test Substance: Crotonaldehyde

Purity: 92.7% **CAS# or LOT#:** CAS #4170-30-3; Lot #7-92

Additional Comments and/or Modifications:

Joseph G. Lorenz, July 27, 1992
Sponsor Approval Date

TO BE COMPLETED BY SLI PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc. SLI Study Number: 1852.0692.6103.130

Study Director: Arthur E. Pratt

Test Concentrations: 1.5, 0.75, 0.38, 0.19, 0.094 mg A.I./L

Solvent Used: Nanopure water CAS# or LOT#: NA

Proposed Schedule: (Start) 8/24/92 (Completion) 9/14/92

Additional Comments and/or Modifications:

Arthur E. Pratt, 7/28/92
Study Director Date

PROTOCOL FOR CONDUCTING A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH *DAPHNIA MAGNA* FOLLOWING TSCA TEST STANDARD NO. 797-1330.

OBJECTIVE

This document describes standard toxicity test procedures used in the performance of a life cycle toxicity test with *Daphnia magna* followed at the Environmental Toxicology & Chemistry Division of Springborn Laboratories, Inc., Wareham, Massachusetts. The procedure closely follows the TSCA Test Standard § 797.1330 (U.S. Environmental Protection Agency. 1985, 1987. *Toxic Substances Control Act Test Guidelines,* Federal Register 50(188), September 27, 1985. Amended, May, 1987), and shall conform to the consent order established between Eastman Kodak Company and U.S. EPA entitled "Testing Consent Order, Crotonaldehyde" (Docket # OPTS 42108). The modified test standard associated with the consent order (§ 797.1330) is presented in Appendix I.

Life cycle toxicity tests are conducted in order to obtain an estimate of the MATC (Maximum Acceptable Toxicant Concentration). The MATC is defined as the highest toxicant concentration not causing a statistically significant effect when compared to controls on the biological parameters measured (adult immobilization, total offspring per adult and immobilized offspring per adult) during continuous chronic exposure. This value is presented as a range encompassing the highest "no effect" concentration (NOEC) and the lowest observed effect concentration (LOEC).

MATERIALS AND METHODS

TEST ORGANISMS:

1. Species. The water flea, *Daphnia magna*, is the species used in this test. Test organisms are ≤ 24 hours old at the initiation of the test. Daphnids are obtained by removing all immature daphnids from the culture vessel, thus isolating sexually mature daphnids 24 hours prior to initiating the test. Young produced by these isolated organisms are subsequently used for test initiation. Daphnids are not used if the culture contains any ephippia, if adults in the cultures do not produce young before day 12, if adults in the cultures do not produce an average of at least 3 young per adult per day over the 7 day period prior to the test, if more than 20% of the culture stock die in the two days preceding the start of the test, or if organisms have been used in any portion of a previous test either in a treatment or control vessel.

2. Source. *D. magna* cultures are maintained at Springborn Laboratories, Inc. Daphnids are cultured in 2-L glass vessels containing 1 L of water in facilities with background colors and light intensity similar to those of the testing area. Water used to culture the daphnids is prepared in the same manner and has the same characteristics as described

for dilution water. Culture water is maintained at test temperature 20 ± 2°C for at least 48 hours prior to initiation of the test. Each culture vessel is cleaned once weekly.

3. Feeding. While being maintained in culture prior to the test, organisms are fed once daily a combination of trout food suspension and a unicellular green algae, *Ankistrodesmus falcatus*. During the 21-day test, test organisms are fed three times daily on weekdays, and twice daily on weekends/holidays. They are fed a combination of a trout food suspension and *Ankistrodesmus falcatus*, supplemented with Selco^{R}, a saturated fatty acid additive. The food solution contains 5 mg/mL trout food suspension, 0.60 mg/mL Selco, and approximately 4×10^{7} cells/mL algae. At each feeding each replicate chamber is fed 2 mL of the trout food suspension, 0.5 mL of the Selco solution and 3 mL of the *Ankistrodesmus falcatus* suspension. Routine analyses are conducted on the food source to ensure the absence of contamination which would be expected to alter the results of the study.

4. Handling. Wide-bore pipets, with inside diameter greater than 5 mm, are used to transfer the daphnids, taking care to minimize possible stress due to handling. Daphnids that are damaged or dropped during transfer are not used. Care is taken to introduce the daphnids below the surface of the test solution so as not to trap air under the carapace.

PHYSICAL SYSTEM:

1. Test Containers. The test chambers used in the flow-through chronic test are 1.6 L or 2-L clear glass battery jars which are chemically clean. Each jar has a 3 x 8 cm notch cut out on the upper edge, or two 2 cm holes drilled in the sides of the jar and both the notches and holes are covered with Nitex^{R} 40-mesh screen for drainage. The test solution volume is thus maintained at approximately 1.4 to 1.8 liters. The test containers are covered with a plastic sheet to prevent dust from falling in the test solution. Testing facilities (i.e., laboratory area) are well ventilated and free of fumes and other disturbances that may affect the test organisms.

2. Cleaning. The diluter is disassembled and cleaned before use. The water cell is brushed and siphoned in place. The chemical cells, mixing chamber, splitters, delivery tubes and test vessels are removed from the unit and washed with hot water and soap, then cleaned by an appropriate method to remove residue of the test material previously used (i.e., acid to remove metal and bases; detergent and organic solvents to remove organic compounds) and rinsed several times with diluent water. The diluter is then reconstructed and allowed to cycle for at least 24 hours for further rinsing. During the test, the test vessels are cleaned with water and soap at a minimum of three times weekly.

3. Dilution Water. Dilution water consists of hard fortified unchlorinated well water with a total hardness of 160 to 180 mg/L $CaCO_3$. The well water (total hardness of approximately 30 mg/L as $CaCO_3$) is fortified according to the formulation for hard water presented in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975). Hard water is used in the chronic daphnid test, because the survival and reproduction of *D. magna* is enhanced under these conditions. Dilution water is filtered through an amberlite XAD-7 resin column and an activated carbon bed

prior to delivery to the diluter. The column is about 15 cm long and 1.6 cm wide. This filtration effectively removes any potential organic contaminants from the water. The resin is replaced in the column prior to initiation of each study.

Quality of the dilution water used to conduct daphnid chronic tests is judged by the ability of the daphnid cultures to survive and reproduce in the water free of stress. The dilution water is prepared in 1,900-L batches. New batches of diluent water are prepared when the previous batch is exhausted, when a water quality parameter (total hardness, alkalinity, etc.) has varied from the normal ranges, or after two weeks of holding. The diluent water is aerated with an air pump and air stones to bring the pH and dissolved gases into equilibrium with the atmosphere. Fiberglass containers are used to hold the diluent water, and water is pumped from this holding tank to the diluter. At least twice each year analyses of representative samples of dilution water source are conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the daphnids. A historical summary is presented in Appendix II. TOC, COD, particulate matter and unionized ammonia, analyses are conducted once a month in the dilution water. The TOC concentration has ranged from 0.32 to 1.8 mg/L in the dilution water source during the last 24 months.

Total hardness, total alkalinity, pH and specific conductance of the diluent water are monitored on each batch prior to use to assure that these parameters are within the normal acceptable ranges. Total hardness and alkalinity are determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Ranges for these parameters generally are: total hardness, 160 - 180 mg/L $CaCO_3$; alkalinity, 110 - 130 mg/L $CaCO_3$; specific conductance, 400 to 600 µmhos/cm; and pH, 7.9 - 8.3.

4. Diluter. A 200-mL proportional diluter (e.g., Mount and Brungs, 1967), with a 0.5 dilution factor is employed to deliver five toxicant concentrations, a control, and a solvent control, if necessary, to four replicate jars. Each dose level is twice the next lower concentration of the test material. The exposure system is constructed entirely of glass, silicone, and nylon.

One of the following toxicant delivery systems is used: the gas-tight syringe injector metering device (most frequently used) or the metering pump/predilution chamber system. Factors considered in the selection of the appropriate toxicant delivery system are the solubility of the material in water under test conditions, and the range of concentrations tested.

A flow-splitting chamber is used between the diluter cells and the four replicate vessels to promote mixing of the toxicant solution and diluent water and to equally split the test solution between the test vessels. Four separate 1 mm (I.D.) glass capillary tubes exit each splitter cell and enter individual delivery tubes which transfer the test solution to each replicate vessel. The capillary tubes baffle the flow of the test solution and minimize turbulence in the test vessels.

The calibration of the diluter system is checked prior to test initiation, weekly during the study, and after test termination. Calibration includes determining the flow rate through each chamber and the proportion of stock solution to dilution water delivered to each

chamber. If there is any indication during the test that the diluter calibration has changed (e.g., diluter malfunction or unexplained differences in dissolved oxygen concentration or temperature in the vessels), calibration of the necessary diluter components is checked. A complete check of diluter functioning is made at least once daily. A test is not started until the diluter and toxicant delivery device have been observed to be properly functioning for at least 48 hours prior to the test. During a test, the flow rates vary no more than 10% from one replicate test chamber to another.

5. Flow Rate. Delivery rates of the test material to each vessel is equal to approximately six test vessel volumes per day. This flow rate is adequate to maintain good water quality and does not stress the organisms due to excessive turbulence.

6. Replication. Four replicates are maintained with each test concentration and control. Each replicate vessel contains 10 individuals, a total of 40 daphnids per concentration or control.

CHEMICAL SYSTEM:

1. Test Material. Upon arrival at Springborn Laboratories, Inc., the external packaging of the test material is inspected for damage. The packaging is removed and the primary storage container is also inspected for leakage or damage. The sample identity is recorded and the material is stored in the dark at 2 - 4°C until used. Exposure of the test material to air should be avoided to minimize the potential for oxidation. The test material should be kept in a tightly sealed container and any head space should be purged of air using nitrogen or helium.

2. Toxicant Concentration Selection. Toxicant concentrations for the chronic toxicity test are selected based on information provided by the Sponsor, from a 48-hour EC50 value, or from a preliminary range-finder test with *D. magna*. The range of concentrations selected for the definitive test is intended to include concentration response curves, EC_{50} values and MATC, but due to the nature of some materials, EC_{50} values may be estimated as greater than the highest treatment level tested. Five concentrations and one control are used for each definitive test, 40 daphnids exposed to each concentration (see above). A dilution ratio of 2 is used.

3. Stock Preparation. Test material is weighed on an analytical balance for which a calibration log is maintained. A Chemical Usage Log is also maintained in which the amount, the date, the intended use and the user's initials are recorded each time test material is used. The stock solution is prepared according to the following formula:

$$\text{Stock concentration} = \frac{\text{H.C. x M.C.}}{\text{B.D. x (\% A.I. } \div 100)}$$

where:

H.C. = high concentration (mg/L)

M.C. = mixing chamber volume (L)

B.D. = bird or syringe delivery (mL)

A.I. = % active ingredient

4. Carrier Solvent - The test material stock solutions are prepared in dilution water without the use of a solvent (carrier).

EXPERIMENTAL PROCEDURE:

1. Test Initiation. The experimental design of this test incorporates four replicate glass vessels per treatment. Each vessel contains approximately 1.4 to 1.8-L of test solution and 10 impartially-selected daphnids. The four vessels for each treatment are arranged in rows, with randomization of the position of each vessel within the row. Positions of rows are also randomly assigned. Daphnids are exposed to five concentrations of the test material. Additionally, a set of control vessels consisting of dilution water containing no test material is maintained. At the initiation of the study, each test concentration is prepared as outlined above (see Chemical System). Daphnids are impartially selected and distributed to each of 24 unlabelled intermediate vessels (i.e., 100 mL beakers) containing 40 mL dilution water and several drops of algae food solution. The daphnids are impartially added, two at a time to each intermediate vessel until each vessel contains two organisms. The process is repeated until each vessel contains 10 organisms. The daphnids are then introduced into the exposure replicate vessels, starting from the control and working through the highest treatment level, by impartially selecting one of the unlabelled intermediate vessels containing 10 organisms, and gently pipetting them one at a time under the surface of the test solution. Food solutions are added to the exposure solutions prior to introduction of the daphnids.

2. Sampling and Measurements of Toxicant Concentrations - The concentration of test substance will be measured only in the diluter stock solution. Triplicate samples of the stock solution and a single sample of a reagent blank are taken at least twice prior to the initiation of the test, at the initiation of the test (day 0), and weekly thereafter for determination of toxicant concentration. Three quality control samples are prepared at each sampling interval and remain with the set of samples through extraction, storage and analysis. These samples are prepared in diluent water at test material concentrations similar to the stock concentration. Results of these analyses are indicative of the relative accuracy of the analytical methodologies for each sampling period. Samples are extracted immediately after sampling.

3. Analytical Method-Sample and Stock Stability Studies - The analytical method for the test substance shall be validated prior to beginning the study. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

Prior to initiating the study, the stability of the toxicant stock solution is established. A stock solution consisting of the same concentration of test material and solvent to be used in the study is prepared. Two aliquots of the stock are removed and analyzed immediately. The stock solution is retained for a minimum of one week under the same conditions as the diluter stock solution (e.g., ambient temperature, laboratory light); then two additional aliquots are removed and analyzed.

4. Photoperiod. All tests are conducted in a light-controlled laboratory. The tests are illuminated to a light intensity of 30 - 100 footcandles using a combination of fluorescent bulbs. A 16-hour light, 8-hour dark photoperiod is maintained by an automatic timer, with a 20 minute transition period between light and dark phases.

5. Measurement of Water Quality Parameters in Exposure Solutions. At test initiation and weekly thereafter, temperature, pH and dissolved oxygen concentration are measured and recorded in each replicate vessel of all test concentrations and control. On the remaining days (i.e., days 2-6, day 8-13, etc.), temperature, pH and dissolved oxygen concentration are determined in one replicate vessel of all test concentrations and the control. Total hardness, alkalinity and specific conductance are determined at test initiation and weekly thereafter, in one replicate of the high and low test concentration and control. Measurements taken in one replicate are alternated among the replicate vessels.

6. Dissolved Oxygen. Total dissolved oxygen is not allowed to drop below 60% or exceed 105% of saturation for the duration of the test. Aeration (with oil free air) would be initiated as a last resort to raise and maintain the dissolved oxygen concentration at or above 60% of saturation.

7. Temperature. Water temperature of the test solutions is maintained at 20 ± 2 °C by conducting the test in a temperature-controlled room maintained at the appropriate test temperature, or in a constant-temperature water bath.

8. Biological Data. The number of immobilized daphnids in each test vessel is recorded on days 1, 2, 4, 7, 14, and 21 of the in-life test. Immobilization is defined by lack of movement by daphnids except for minor activity of appendages. Immobilization and reproduction is determined by counting and observing adults as they are carefully pipetted from the exposure vessel to a 100 mL beaker containing approximately 50 mL of the respective test solution. After removing the adult daphnids, the exposure solution is then filtered through a fine mesh net into a holding vessel to remove offspring. Offspring are removed from the net by inverting and dipping the net into a 100 mL beaker containing dilution water. These 100 mL beakers containing the offspring are put aside and counted after adult immobilization has been determined. The exposure vessel is then cleaned and carefully rinsed with water. The original test solution is then returned and the beaker containing the adult daphnids is lowered in the exposure vessel and slowly tipped to allow the water to flow slowly into the test vessel and allow the daphnids to swim out. Following day 7 of the test adult immobilization and offspring produced are counted and removed at a minimum of three times per week, e.g. Mondays, Wednesdays and Fridays, and observations of abnormal behavior are made. The number of immobilized offspring and the time to first brood release are recorded for each replicate test vessel. In addition, whenever test organisms are observed, characteristics of the test

solutions are also observed and recorded, e.g., precipitated materials, cloudiness, etc. The test is terminated following 21 days of exposure.

9. Acceptance Criteria - A test is considered unacceptable if more than 20% of control daphnids appear to be immobilized, stressed or diseased during the test; each control daphnid living the full 21 days produces an average of less than 60 young; and/or any ephippia are produced by control animals.

STATISTICAL ANALYSES:

1. Endpoints - The endpoints used for the determination of significant effects by statistical evaluation include the number of immobilized adults, total offspring per adult, and immobilized offspring per adult.

2. Statistical Methods - If a solvent is used as carrier for the test material, and the concentration of the carrier solvent in the solvent control caused a statistically significant effect, either enhancement or reduction (analysis of variance, $P \leq 0.05$), the treatment data are compared to that of the solvent control. If the solvent concentration did not affect the measured or calculated endpoint, both controls (dilution water, solvent control) are pooled for the data analysis. The method used to evaluate the results of the life cycle daphnid test is *Williams' Test* (Williams, 1971, 1972) coupled with *Bartlett's test* for determination of homogeneity of variances. If necessary, mean values are transformed using square root, arcsine square root, or log conversion procedures. If, after appropriate transformation procedures have been applied to the data, *Bartlett's test* still fails to demonstrate homogeneity of variances, then non-parametric methods are used to compare sample means, such as the *Kruskal-Wallis and Steel's One-Many Rank test*. The maximum concentration at which a test material can be present and not be toxic to the test organism is expressed as the maximum acceptable toxicant concentration (MATC).

3. MATC - The MATC is determined for the most sensitive test criteria measured (number of adult daphnids immobilized, number of offspring per adult and number of immobilized offspring per adult), by taking the geometric mean of the limits set by the lowest test concentration that shows a statistically significant effect at the 95% level of certainty (lowest observed effect concentration, LOEC) and the highest test concentration that shows no statistically significant difference from the control (highest no observed effect concentration, NOEC).

4. Transformations - Transformation of data is limited to data representing endpoint estimates obtained as a proportion (e.g., survival). Prior to analyzing data of this type, the observed proportion in each tank is transformed by using the arcsine square-root transformation.

5. EC50 - Whenever sufficient concentration-response data are generated, EC_{50} values and associated 95% confidence limits for adult immobilization are determined for 7, 14 and 21 days of exposure. The EC50 is the estimated nominal concentration of the test material in dilution water which produces 50% immobility in the test populations of

daphnids at the stated times of exposure. The computer program utilized produces EC50 values using three statistical methods: probit analysis, moving average method, and binomial probability. The method selected and reported is determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An EC50 value cannot be calculated if the data derived are insufficient according to any of the three statistical methods. The method provides values of the slope, including 95% confidence intervals, for the probit analysis, as well as appropriate statistical tests to evaluate goodness-of-fit.

REPORTING

The raw data and final draft of the report are reviewed by the Quality Assurance Unit and Study Director. All values of chemical and water quality measurements are reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the study sponsor for review. Upon acceptance by the sponsor, three copies of the final report will be submitted. All reports include, but are not limited to, the following information:

* Springborn Laboratories, Inc., report and project numbers.
* Identification of Study Sponsor.
* Laboratory and site, the dates of testing and a list of personnel involved in the study, i.e., Study Director, Principal Investigator and technicians, and identification of the Quality Assurance Unit.
* All information pertaining to the test material which appears on the sample bottle, e.g., its source, percent active ingredient, physical properties, Sponsor's test material I.D., and sample number.
* Characterization and origin of the dilution water.
* Scientific name of test organisms, method of verification, source, age , origin of brood stock, and culture information, acclimation procedures and conditions and feeding history. Historical cultures records will be used as documentation of colony robustness.
* A description of the experimental design, the test chambers and depth and volume of the solution in chambers the flow rate as volume addition per 24 hours, the procedure for test initiation, the number of organisms per treatment, the number of replicate chambers per treatment, the biomass loading rate, light intensity and photoperiod, and a description of the test substance delivery system.
* Detailed information on feeding of daphnids during toxicity test, including type of food used, its source, feeding frequency and results of analysis (i.e., concentration) for contaminants.

* Definition of criteria used to determine the sublethal effects, and general observations on nonquantifiable effects.

* Test temperatures, dissolved oxygen concentration, and pH; as well as specific conductance, total alkalinity and total hardness measured.

* Means and standard deviations of measured concentrations of the test material in the stock solutions, as well as nominal test concentrations.

* Description of, or reference to chemical and statistical procedures applied, description of stock solution preparation including method validation and reagent blanks.

* Percentage of parental and offspring daphnids that were immobilized, displayed any abnormal behavior or appearance in the controls and in each treatment at each observation period, in tabular form.

* The NOEC, LOEC and MATC values of all effects criteria used, and the level of certainty applied to the statistical analyses. These calculations will be made using the nominal test concentrations.

* The 7, 14, 21-day EC50 with 95 percent confidence limits. (Based on nominal concentrations of the test material)

* Reference to the location where the raw data are stored.

* Deviations from the protocol not addressed in protocol amendments will be listed, together with a discussion of the impact on the study and signed by the Study Director.

* Good Laboratory Practice (GLP) compliance statement signed by the Study Director.

* Dates of Quality Assurance Audits, signed by the QA Unit.

SPECIAL PROVISIONS

GOOD LABORATORY PRACTICE STANDARDS (GLP): All test procedures, documentation, records, and reports will comply with the U. S. Environmental Protection Agency's Good Laboratory Practice Standards as promulgated under the Toxic Substances Control Act Part 192 (*FEDERAL REGISTER*, Part III, 17 August, 1989)

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, unless different arrangements are made.

TEST MATERIAL ARCHIVAL: It will be the responsibility of the Sponsor to retain a reserve sample of each batch of the test substance, as required by EPA GLP (US EPA, 1989) for studies of greater than 4 weeks duration.

REFERENCES

APHA, AWWA, WPCF. 1989. *Standard Methods for the Examination of Water and Wastewater.* 17th Edition, Washington, DC. 2168 pp.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Research 1: 20-29.

U.S. EPA. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009). 61 pp.

U.S. Environmental Protection Agency. 1985, 1987. *Toxic Substances Control Act Test Guidelines.* Federal Register. Vol. 50, No. 188, September 27, 1985, amended, May, 1987. "§ 797.1330. Daphnid Chronic Toxicity Test."

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27: 103-117.

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APPENDIX I

**TESTING CONSENT ORDER, CROTONALDEHYDE
(DOCKET # OPTS 42108)**

SECTION 797.1330 DAPHNID CHRONIC TOXICITY TEST

Section 797.1330 Daphnid chronic toxicity test.

(a) Purpose. This guideline is intended for use in developing data on the chronic toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003. 15 U.S.C. 2601 et seq.). This guideline prescribes a chronic toxicity test in which daphnids are exposed to a chemical in a renewal ~~{or a flow-through}~~ system. The United States Environmental Protection Agency will use data from this test in assessing the hazard a chemical may present to the aquatic environment.

(b) Definitions. The definitions in section 3 of the Toxic Substances Control Act (TSCA), and the definitions in Part 792 Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

(1) "Brood stock" means the animals which are cultured to produce test organisms through reproduction.

(2) "Chronic toxicity test" means a method used to determine the concentration of a substance in water that produces an adverse effect on a test organism over an extended period of time. In this test guideline, mortality and reproduction ~~{(and optionally, growth)}~~ are the criteria of toxicity.

(3) "EC_{50}" means that experimentally derived concentration of test substance in dilution water that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time. In this guideline, the effect measured is immobilization. [EC_{50} VALUE IS CALCULATED BY MEANS OF AN ANOVA APPLIED TO DATA ON YOUNG PRODUCED.]

(4) "Ephippium" means a resting egg which develops under the carapace in response to stress conditions in daphnids.

~~{(5) "Flow-through" means a continuous or intermittent passage of test solution [TEST SUBSTANCE] or dilution water through a test chamber or culture tank} with no recycling.~~

~~{(6)~~ [(5)]] "Immobilization" means the lack of movement by daphnids except for minor activity of the appendages.

~~{(7)~~ [(6)]] "Loading" means the ratio of daphnid biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber at a point in time or passing through the test chamber during a specific interval.

~~{(8)~~ [(7)]] "MATC (Maximum Acceptable Toxicant Concentration)" means the maximum concentration at which a chemical can be present and not be toxic to the test organism.

~~((9)~~ [(8)]] "Renewal system" means the technique in which test organisms are periodically transferred to fresh test solution of the same composition.

(c) Test procedures—(1) Summary of the test. (i) Test chambers are filled with appropriate volumes of ~~(dilution water~~ [THE TEST SOLUTIONS]. ~~In the flow-through test the flow of dilution water through each chamber is then adjusted to the rate desired. The test substance is introduced into each test chamber. The addition of test substance in the flow through system is done at a rate which is sufficient to establish and maintain the desired concentration of test substance in the test chamber.~~)

(ii) The test is started within 30 minutes after the test substance has been added and uniformly distributed in the test chambers in the renewal test (~~or after the concentration of test substance in each test chamber of the flow-through test system reaches the prescribed level and remains stable~~). At the initiation of the test, daphnids which have been cultured or acclimated in accordance with the test design, are randomly placed into the test chambers. Daphnids in the test chambers are observed periodically during the test, immobile adults and offspring produced are counted and removed, and the findings are recorded. Dissolved oxygen concentration, pH, temperature, ~~(the concentration of test substance,~~) and other water quality parameters are measured at specified intervals in selected test chambers. Data are collected during the test to determine any significant differences ($p < 0.05$) in immobilization and reproduction as compared to the control.

(2) (Reserved)

(3) Range-finding test. (i) A range-finding test should be conducted to establish test solution concentrations for the definitive test.

(ii) The daphnids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/l), usually under static conditions.

(iii) A minimum of five daphnids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate chronic test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) Definitive test. (i) The purpose of the definitive test is to determine concentration-response curves. EC_{50} values and effects of a chemical on immobilization and reproduction during chronic exposure.

(ii) A minimum of 20 daphnids per concentration shall be exposed to five ~~(or more)~~ concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/l). An equal number of daphnids shall be placed in two or more replicates. The concentration ranges shall be selected to determine the concentration-response curves, EC_{50} values and MATC. ~~(Solutions shall be analyzed for chemical concentration at designated times during the test.)~~

(iii) Every test shall include controls consisting of the same dilution water, conditions, procedures and daphnids from the same population (culture container), except that none of the chemical is added.

(iv) The test duration is 21 days. The test is unacceptable if:

(A) More than 20 percent of the control organisms appear to be immobilized, stressed or diseased during the test.

(B) Each control daphnid living the full 21 days produces an average of less than 60 young.

(C) Any ephippia are produced by control animals.

(v) The number of immobilized daphnids in each chamber shall be recorded on day 21 of the test. After offspring are produced, they shall be counted and removed from the test chambers every 2 or 3 days. [WHENEVER SUFFICIENT DOSE-RESPONSE DATA ARE GENERATED,] Concentration-response curves, EC_{50} values and associated 95 percent confidence limits for adult immobilization shall be determined for ~~day~~ [DAYS 7, 14 AND] 21. A MATC shall be determined for the most sensitive test criteria measured (number of adult animals immobilized, number of young per adult and number of immobilized young per adult).

(vi) In addition to immobility, any abnormal behavior or appearance shall also be reported.

(vii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner as in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(5) (Reserved)

(6) Analytical measurements—(i) Test chemical. {~~Deionized for DISTILLED~~} [DILUENT]} water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

[(ii) THE ANALYTICAL METHOD FOR THE TEST SUBSTANCE SHALL BE VALIDATED PRIOR TO BEGINNING THE TEST. A PROCEDURE SUCH AS USING KNOWN ADDITIONS MAY BE USED. THIS INVOLVES ADDING KNOWN AMOUNTS OF THE TEST SUBSTANCE TO {~~THREE OR MORE SAMPLES OF DILUTION WATER~~ [THE SAME TYPE OF WATER USED TO PREPARE THE STOCK SOLUTIONS]}. THE NOMINAL CONCENTRATION{~~S~~} OF THE TEST SUBSTANCE IN THESE SAMPLES SHOULD {~~SPAN~~ [APPROXIMATE]} THE CONCENTRATION {~~RANGE TO BE USED IN THE TEST. BOTH DISSOLVED TEST SUBSTANCE (THAT WHICH PASSES THROUGH A 0.45 MICRON FILTER) AND TOTAL TEST SUBSTANCE SHALL BE MEASURED IN EACH SAMPLE. IF THE MEASURED CONCENTRATIONS OF DISSOLVED TEST SUBSTANCE ARE GREATER THAN 80% OF THE MEASURED CONCENTRATIONS OF TOTAL TEST SUBSTANCE, THEN ONLY TOTAL TEST SUBSTANCE SHALL BE MEASURED DURING THE TEST. HOWEVER, IF THE MEASURED CONCENTRATIONS OF DISSOLVED TEST SUBSTANCE ARE LESS THAN 80% OF THE MEASURED CONCENTRATIONS OF TOTAL TEST SUBSTANCE, THEN ONLY DISSOLVED TEST SUBSTANCE SHALL BE MEASURED DURING THE TEST~~ [OF THE TEST SOLUTION]}. VALIDATION OF THE ANALYTICAL METHOD SHOULD BE PERFORMED ON AT LEAST TWO SEPARATE DAYS PRIOR TO STARTING THE TEST.]

[(iii) {~~SUBJECT TO CONSTRAINTS ASSOCIATED WITH LIMITS OF DETECTION, ALL DOSE LEVELS WILL BE ANALYZED FOR THE TEST ARTICLE AT LEAST ONCE EVERY SEVEN DAYS. EQUAL ALIQUOTS OF TEST ARTICLE SOLUTION (OR CONTROL SOLUTION) MAY BE REMOVED FROM REPLICATE TEST VESSELS AND COMBINED FOR ANALYSIS.~~} IN ADDITION TO ANALYZING {~~SAMPLES OF TEST~~ [THE STOCK]} SOLUTION, AT LEAST ONE REAGENT BLANK, CONTAINING ALL REAGENTS USED, SHOULD ALSO BE ANALYZED.] {[THE STOCK SOLUTION WILL BE ANALYZED FOR THE TEST ARTICLE AT LEAST ONCE EVERY SEVEN DAYS.]}

{~~[(iv) FILTERS AND THEIR HOLDERS USED FOR DETERMINING THE DISSOLVED TEST SUBSTANCE CONCENTRATIONS SHOULD BE PREWASHED WITH SEVERAL VOLUMES OF DISTILLED WATER OR DILUTION WATER AND UNDERGO A FINAL RINSE WITH TEST SOLUTION. GLASS OR STAINLESS STEEL FILTER HOLDERS ARE BEST FOR ORGANIC SUBSTANCES, WHILE PLASTIC HOLDERS ARE BEST FOR METALS. THE SAMPLE SHOULD BE FILTERED WITHIN 30 MINUTES AFTER IT IS TAKEN FROM THE TEST CHAMBER.]~~}

~~[(iii)~~ {~~[(v)~~ [(iv)]}. Numerical. The number of immobilized adults, total offspring per adult and immobilized offspring per adult shall be counted during each test. Appropriate statistical analyses should provide a goodness-of-fit determination for the adult immobilization concentration-response curves calculated on day 21. A 21-day EC_{50}, based on adult immobilization and corresponding 95 percent confidence intervals, shall also be calculated.

Appropriate statistical tests (e.g., analysis of variance, mean separation test) should be used to test for significant chemical effects on chronic test criteria (cumulative number of immobilized adults, cumulative number of offspring per adult and cumulative number of immobilized offspring per adult) on day 21. An MATC shall be calculated using these chronic test criteria.

(d) Test conditions—(1) Test species—(i) Selection (A) The cladocerans, Daphnia magna ~~or D. pulex, are~~ [IS] the species to be used in this test. ~~{Either species can be utilized for testing of a particular chemical.}~~ The species identity of the test organisms should be verified using appropriate systematic keys.

(B) First instar daphnids, <24 hours old, are to be used to start the test.

(ii) Acquisition. (A) Daphnids to be used in chronic toxicity tests should be cultured at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test shall have originated from the same culture population. [DAPHNID COLONY RECORDS WILL BE USED AS DOCUMENTATION OF COLONY ROBUSTNESS.]

(B) Daphnids shall not be used for a test if:

(1) Cultures contain ephippia.

(2) Adults in the cultures do not produce young before day 12.

(3) More than 20 percent of the culture stock die in the 2 days preceding the test.

(4) Adults in the culture do not produce an average of at least 3 young per adult per day over the 7-day period prior to the test.

(5) Daphnids have been used in any portion of a previous test either in a treatment or in a control.

(iii) Feeding. (A) During the test the daphnids shall be fed the same diet and with the same frequency as that used for culturing and acclimation. All treatments and control(s) shall receive, as near as reasonably possible, the same ration of food on a per-animal basis.

(B) The food concentration depends on the type used. Food concentrations should be sufficient to support normal growth and development and to allow for asexual (parthenogenic) reproduction.

For automatic feeding devices, a suggested rate is 5 to 7 mg food (either solids or algal cells, dry weight) per liter dilution water or test solution. For manual once-a-day feeding, a suggested rate is 15 mg food (dry weight) per liter dilution water or test solution.

(iv) Loading. The number of test organisms placed in a test chamber shall not affect test results. Loading shall not exceed 40 daphnids per liter in the renewal system. ~~(In the flow-through test, loading limits will vary depending on the flow rate of the dilution water.)~~ Loading shall not cause the dissolved oxygen concentration to fall below the recommended level.

(v) Care and handling of test organisms. (A) Daphnids should be cultured in dilution water under similar environmental conditions to those used in the test. A variety of foods have been demonstrated to be adequate for daphnid culture. They include algae, yeasts and a variety of mixtures.

(B) Organisms should be handled as little as possible. When handling is necessary it should be done as gently, carefully and quickly as possible. During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage and mortality. Dead and abnormal individuals shall be discarded. Organisms that touch dry surfaces or are dropped or injured during handling should be discarded.

(C) Smooth glass tubes (I.D. greater than 5 mm) equipped with a rubber bulb can be used for transferring daphnids with minimal culture media carry-over.

(D) Care should be exercised to introduce the daphnids below the surface of any solution so as not to trap air under the carapace.

(vi) Acclimation. (A) Brood daphnids shall be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test. This is easily accomplished by culturing them in the dilution water at the test temperature. During acclimation, daphnids shall be fed the same food as will be used for the definitive test.

(B) During culturing and acclimation to the dilution water, daphnids should be maintained in facilities with background colors and light intensities similar to those of the testing area.

(2) Facilities—(i) General. (A) Facilities needed to perform this test include:

(1) Containers for culturing and acclimating daphnids.

(2) A mechanism for controlling and maintaining the water temperature during the culturing, acclimation and test periods.

(3) Apparatus for straining particulate matter, removing gas bubbles, or aerating the water when water supplies contain particulate matter, gas bubbles, or insufficient dissolved oxygen, respectively.

(4) An apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period.

(5) An apparatus to introduce food if continuous or intermittent feeding is used.

~~{(c) In addition, the flow-through test shall contain appropriate test chambers in which to expose daphnids to the test substance and an appropriate test substance delivery system.}~~

(B) Facilities should be well ventilated and free of fumes and other disturbances that may affect the test organisms.

(ii) Test chambers. (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from the dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(B) For renewal tests, daphnids can be conveniently exposed to the test solution in 250 ml beakers or other suitable containers.

~~{(C) For flow-through tests daphnids can be exposed in glass or stainless steel containers with stainless steel or nylon screen bottoms. Such containers shall be suspended in the test chamber in a manner to ensure that the test solution flows regularly into and out of the container and that the daphnids are always submerged in at least 5 centimeters of test solution. Test chambers can be constructed using 250-ml beakers or other suitable containers equipped with screened overflow holes, standpipes or V-shaped notches.}~~

~~{(D)}~~ [(C)] Test chambers shall be ~~loosely~~ covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(iii) Test substance delivery system. ~~{(A) In the flow-through test,}~~ ~~proportional diluters,~~ ~~{metering pump systems or other suitable systems should be used to deliver the test substance to the test chambers.}~~

~~{(f) The test substance delivery system used shall be calibrated before and after each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24 hour flow rate through a test chamber shall be equal to at least five times the volume of the test chamber. During a test, the flow rates shall not vary more than 10 percent from any one test chamber to another or from one time to any other.~~ [(A)]} For the renewal test, test substance dilution water shall be completely replaced ~~{at least once every 7 days~~ [DAILY]}.

(iv) Dilution water. (A) Surface or ground water, reconstituted water, or dechlorinated tap water are acceptable as dilution water if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the following specifications:

Substance	Maximum Concentration	
Particulate matter	20	mg/l.
Total organic carbon or	~~2~~ [3]	mg/l.
Chemical oxygen demand	5	mg/l.
Un-ionized ammonia	20	µg/l.
Residual chlorine	~~1~~ [10]	µg/l.
Total organophosphorus pesticides	50	ng/l.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	50	ng/l.
or organic chlorine	25	ng/l.

(B) The water quality characteristics listed above shall be measured at least twice a year or when it is suspected that these characteristics may have changed significantly. If dechlorinated tap water is used, ~~daily~~ chlorine analysis shall be performed [AT EACH RENEWAL]. [FOR THE ANALYTICAL REQUIREMENTS OF THE DILUENT WATER, THE ATTACHED AGGREGATE HISTORICAL DATA SUMMARY WILL BE SUBSTITUTED. THE MEASURED RESIDUAL CHLORINE SHOULD BE LESS THAN 0.01 mg/L.]

(C) If the diluent water is from a ground or surface water source, conductivity and total organic carbon (TOC) or chemical oxygen demand (COD) should be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon filtered deionized water with a conductivity of less than 1 microohm/cm is acceptable as the diluent for making reconstituted water.

~~{(D) If the test substance is not soluble in water an appropriate carrier should be used.}~~

(v) Cleaning of test system. All test equipment and test chambers shall be cleaned before each use following standard laboratory procedures. Cleaning of test chambers may be necessary during the testing period.

(3) Test parameters. (i) Environmental conditions of the water contained in test chambers should be maintained as specified in this paragraph:

(A) The test temperature shall be 20°C. Excursions from the test temperature shall be no greater than ± 2°C.

(B) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, shall be done before the addition of the test substance. All treatment and control chambers shall be given the same aeration treatment.

(C) Photoperiod of 16-hours light and 8-hours darkness {[WITH A 20-MINUTE TRANSITION PERIOD BETWEEN LIGHT AND DARK PHASES]}.

(ii) Additional measurements include:

(A) The concentration of the test substance in the {~~chambers~~ [STOCK SOLUTION]} shall be measured during the test {[AT THE START OF THE EXPOSURE AND AT LEAST ONCE EVERY SEVEN DAYS THEREAFTER]}.

~~{(B) At a minimum, the concentration of test substance should be measured as follows:~~

~~(1) In each chamber before the test.~~

~~(2) In each chamber on days 7, 14, and 21 of the test.~~

~~(3) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.}~~

{~~(C)~~ [(B)]} The dissolved oxygen concentration, temperature and pH shall be measured at the beginning of the test and on days 7, 14, and 21 in at least two chambers of the high, middle, low, and control test concentrations.

(e) Reporting. The sponsor shall submit to the U.S. Environmental Protection Agency all data developed by the test that are suggestive or predictive of chronic toxicity and all associated toxicologic manifestations.

In addition to the reporting requirements prescribed in the Part 792—Good Laboratory Practice Standards of this chapter the reporting of test data shall include the following:

(1) The name of the test, sponsor, testing laboratory, study director, principal investigator, and dates of testing.

(2) A detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers or other additives used and their concentrations.

(3) The source of the dilution water, its chemical characteristics (e.g., conductivity, hardness, pH), and a description of any pretreatment.

(4) Detailed information about the daphnids used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture methods. The age of the daphnids used in the test shall be reported. [DAPHNID COLONY RECORDS WILL BE USED AS DOCUMENTATION OF COLONY ROBUSTNESS.]

(5) A description of the test chambers, the volume of solution in the chambers, the way the test was begun (e.g., conditioning, test substance additions), the number of test organisms per test chamber, the number of replicates per treatment, the lighting, the renewal process and schedule for the renewal chronic test, ~~{the test substance delivery system and flow rate expressed as volume additions per 24 hours for the flow-through chronic test.}~~ and the method of feeding (manual or continuous) and type of food.

(6) The concentration of the test substance in ~~{test chambers at times designated for renewal and flow-through tests.~~[THE STOCK SOLUTION]}.

(7) The number and percentage of organisms that show any adverse effect in each test chamber at each observation period.

(8) The cumulative adult and offspring immobilization values and the progeny produced at designated observation times, the time (days) to first brood and the number of offspring per adult in the control replicates and in each treatment replicate.

(9) All chemical analyses of ~~water quality and~~ test substance concentrations, including methods, method validations and reagent blanks. [FOR THE ANALYTICAL REQUIREMENTS OF THE DILUENT WATER, THE ATTACHED AGGREGATE HISTORICAL DATA SUMMARY WILL BE SUBSTITUTED. LIMITS OF DETECTION SHALL BE INCLUDED.]

(10) The data records of the culture, acclimation, and test temperatures.

(11) Any deviation from this test guideline, and anything unusual about the test, (e.g., dilution failure, temperature fluctuations).

(12) The MATC to be reported is calculated as a geometric mean between the lowest {~~measured~~ [NOMINAL]} test substance concentration that had a significant ($p < 0.05$) effect and the highest {~~measured~~ [NOMINAL]} test substance concentration that had no significant ($p < 0.05$) effect on day 21 of the test.. The most sensitive of the test criteria (number of adult animals immobilized, the number of young per female and the number of immobilized young per female) is used to calculate the MATC. The criterion selected for MATC computation is the one which exhibits an effect (a statistically significant difference between treatment and control groups: $p < 0.05$) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance, mean separation test) shall be used to test for significant test substance effects. The statistical tests employed and the results of these tests shall be reported.

(13) Concentration-response curves utilizing the {~~average measured~~ [NOMINAL]} test substance concentration{[S]} shall be fitted to cumulative adult immobilization data at 21 days. A statistical test of goodness-of-fit shall be performed and the results reported.

(14) An EC_{50} value based on adult immobilization with corresponding 95 percent confidence limits when sufficient data are present for day 21. These calculations should be made using the {~~average measured~~ [NOMINAL]} concentration{[S]} of the test substance.

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APPENDIX II

HISTORICAL DILUTION WATER ANALYSIS

APPENDIX II

GFT Grab Water Sample*		
Sample Range: 3/29/89 - 1/16/92		
Pesticide Screen I;II;III	Results Received (Range)	Maximum Limit of Quantitation
Alpha BHC	< 0.01 - < 0.02 µg/L	0.02
Beta BHC	< 0.02 - 0.01 µg/L	0.02
Gamma BHC - Lindane	<0.02 - 0.01 µg/L	0.02
Delta BHC	< 0.01 - < 0.02 µg/L	0.02
Heptachlor	< 0.01 - < 0.02 µg/L	0.02
Aldrin	< 0.01 - < 0.02 µg/L	0.02
Heptachlor Epoxide	< 0.01 - < 0.02 ug/L	0.02
DDE	< 0.01 - < 0.02 µg/L	0.02
DDD	< 0.01 - < 0.02 µg/L	0.02
DDT	< 0.01 - < 0.02 µg/L	0.02
HCB	< 0.01 - < 0.02 µg/L	0.02
Mirex	< 0.01 - < 0.02 µg/L	0.02
Methoxychlor	< 0.05 - < 0.2 µg/L	0.1
Dieldrin	< 0.01 - < 0.02 µg/L	0.02
Endrin	< 0.01 - < 0.02 µg/L	0.02
Telodrin	< 0.01 - < 0.02 µg/L	0.02
Chlordane	< 0.05 - < 0.1 µg/L	0.1
Toxaphene	< 0.1 - < 2. µg/L	2.
PCB's	< 0.2 - < 2. µg/L	2.
Ronnel	< 0.01 - < 0.02 µg/L	0.02
Ethion	< 0.02 - < 0.05 µg/L	0.05
Trithion	< 0.05 - < 0.1 µg/L	0.1
Diazinon	< 0.1 - < 0.5 µg/L	0.5
Methyl Parathion	< 0.02 - < 0.1 µg/L	0.1
Ethyl Parathion	< 0.05 - < 0.1 µg/L	0.1
Malathion	< 0.05 - < 0.2 µg/L	0.2
Endosulfan I	< 0.01 - < 0.02 µg/L	0.02
Endosulfan II	< 0.01 - < 0.02 µg/L	0.02
Endosulfan Sulfate	< 0.03 - < 0.1 µg/L	0.1
* Analyzed by Lancaster Laboratories, Inc.		

Report No. 92-10-4473

GFT Grab Water Sample*		
Sample Range: 3/2/89 - 1/16/92		
ICP Metals, Screen II	Results Received (Range)	Maximum Limit of Quantitation
Pesticide Screen I,II	attached	
Mercury	< 0.0005 mg/L	0.0005
Arsenic	< 0.05 mg/L	0.05
Selenium	< 0.05 mg/L	0.05
Boron	< 0.005 - < 0.05 mg/L	0.05
Thallium	< 0.1 mg/L	0.1
Aluminum	< 0.1 - < 0.2 mg/L	0.2
Antimony	< 0.05 mg/L	0.05
Barium	< 0.1 - < 0.2 mg/L	0.2
Beryllium	< 0.005 - 0.005 mg/L	0.005
Cadmium	< 0.005 - < 0.05 mg/L	0.005
Calcium	2.3 - 8.7 mg/L	0.5
Chromium	< 0.05 mg/L	0.05
Cobalt	< 0.05 mg/L	0.05
Copper	< 0.02 - < 0.05 mg/l	0.05
Iron	< 0.05 - 0.1 mg/L	0.1
Lead	< 0.05 mg/L	0.05
Lithium	< 0.5 mg/L	0.5
Magnesium	1.1 - 2.1 mg/L	0.5
Manganese	< 0.01 - 0.03 mg/L	0.01
Molybdenum	< 0.1 mg/L	0.1
Nickel	< 0.04 - < 0.05 mg/L	0.04
Potassium	0.5 - 1.2 mg/L	0.5
Silicon	4.2 - < 5. mg/L	0.5 - 5**
Silver	< 0.01 - < 0.05 mg/L	0.05
Sodium	5.1 - 12.8 mg/L	0.5
Strontium	< 0.05 mg/L	0.05
Titanium	< 0.05 mg/L	0.05
Vanadium	< 0.05 mg/L	0.05
Zinc	< 0.02 - < 0.05 mg/L	0.05
* Analyzed by Lancaster Laboratories, Inc.		
** For 10/30/90 sample, the quantitation limit was increased due to nature of sample matrix		

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Environmental Sciences Division
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PROTOCOL AMENDMENT

AMENDMENT #: 1

DATE: 20 August 1992

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with *Daphnia magna* Following TSCA Test Standard No. 797-1330."

SPECIES: *Daphnia magna*

STUDY SPONSOR: Eastman Kodak Company

TEST MATERIAL: Crotonaldehyde

SLI STUDY NO: 1852.0692.6103.130

AMENDMENT(S): The protocol states that the test material stock solutions are prepared in dilution water without the use of a solvent (carrier). During this study the test material stock solutions are prepared in ASTM Type II water (purified using a Nanopure® system) due to increased stability in this type of water.

Approval Signatures: Arthur E. Putt — 20 August 1992
Arthur E. Putt, SLI Study Director — Date

Joseph W. Gorsuch — 8/21/92
Joseph W. Gorsuch, Sponsor Study Monitor — Date

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PROTOCOL AMENDMENT

AMENDMENT #: 2

DATE: 24 September 1992

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with *Daphnia magna* Following TSCA Test Standard No. 797-1330."

SPECIES: *Daphnia magna*

STUDY SPONSOR: Eastman Kodak Company

TEST MATERIAL: Crotonaldehyde

SLI STUDY NO: 1852.0692.6103.130

AMENDMENT(S): The protocol states that the duration of the study is 21 days. During this study the length of testing will be extended to 28 days. The duration of the exposure period was extended to obtain additional information and to establish effects of chronic exposure to Crotonaldehyde on the survival and reproduction of *Daphnia magna*.

Approval Signatures: Curtis E. Putt 24 September 1992
Arthur E. Putt Date
SLI Study Director

Joseph W. Gorsuch 15 October 1992
Joseph W. Gorsuch Date
Sponsor Study Monitor

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PROTOCOL AMENDMENT

AMENDMENT #: 3

DATE: 28 October 1992

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with *Daphnia magna* Following TSCA Test Standard No. 797-1330."

SPECIES: *Daphnia magna*

STUDY SPONSOR: Eastman Kodak Company

TEST MATERIAL: Crotonaldehyde

SLI STUDY NO: 1852.0692.6103.130

AMENDMENT(S): The cover page of the protocol identifies the nominal test concentrations as 1.5, 0.75, 0.38, 0.19 and 0.094 mg A.I./L. Following the initiation of the study, sponsor supplied information revising the percent active ingredient from 92.7% to 93.8% as Crotonaldehyde (CAS# 4170-30-3; Lot# 7-92). As a result of this change, the revised nominal test concentrations for this study are 1.5, 0.76, 0.38, 0.19, 0.095 mg A.I./L.

Approval Signatures: Arthur E. Putt 10/28/92
Arthur E. Putt — Date
SLI Study Director

Joseph W. Gorsuch 11/13/92
Joseph W. Gorsuch — Date
Sponsor Study Monitor

APPENDIX 4 - FOOD AND DILUTION WATER ANALYSES

Ankistrodesmus Suspension Grab Liquid Sample*		
Date Submitted:4/29/92 Date Reported: 5/11/92		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0 01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Ankistrodesmus Suspension Grab Liquid Sample*		
Date Submitted:4/29/92 Date Reported: 5/11/92		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	< 0.1 mg/l	0.1
Cadmium	< 0.005 mg/l	0.005
Lead	< 0.05 mg/l	0.05
Mercury	0.0004 mg/l	0.0002
* Analyzed by Lancaster Laboratories, Inc.		

Lot #031891 A1 Selco Food Sample*		
Date Submitted:5/22/91 Date Reported: 6/7/91		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0.01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg.kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	< 0.01 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCB's	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.05 mg/kg	0.05
Endosulfan I	< 0.01 mg/kg	0.01
Endosulfan II	< 0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Lot #031891 A1 Selco Food Sample*		
Date Submitted:5/22/91 Date Reported: 6/7/91		
Analysis	**Result As Received**	**Limit of Quantitation**
Pesticide Screen I;II;III;	attached	
Arsenic	< 0.1 ppm	0.1
Cadmium	< 0.2 ppm	0.2
Lead	< 0.2 ppm	0.2
Mercury	< 0.02 ppm	0.02
* Analyzed by Lancaster Laboratories, Inc.		

Zeigler Brothers, Inc. Salmon Starter*		
Date Submitted:12/13/90 Date Reported: 1/10/91		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0 01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0 01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg.kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	0.04 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCB's	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.2 mg/kg	0.2
Endosulfan I	<0.01 mg/kg	0.01
Endosulfan II	<0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Zeigler Brothers Inc. Salmon Starter*		
Date Submitted:12/13/90 Date Reported: 1/10/91		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	0.5 ppm	0.1
Cadmium	0.15 ppm	0.05
Lead	0.5 ppm	0.1
Mercury	0.03 ppm	0.02
Selenium (fluorometric)	1.1 ppm	0.1
* Analyzed by Lancaster Laboratories, Inc.		

GFT Grab Water Sample*		
Date Collected:6/23/92 Date Reported: 7/9/92		
Analysis	**Result As Received**	**Limit of Quantitation**
Pesticide screen I;II;III	attached	
Mercury	< 0.0002 mg/l	0.0002
Arsenic	< 0.05 mg/l	0.05
Selenium	< 0.05 mg/l	0.05
Boron	< 0.05 mg/l	0.05
Thallium	< 0.1 mg/l	0.1
Aluminum	< 0.2 mg/l	0.2
Antimony	< 0.05 mg/l	0.05
Barium	< 0.2 mg/l	0.2
Beryllium	< 0.005 mg/l	0.005
Cadmium	< 0.005 mg/l	0.005
Calcium	7.4 mg/l	0.5
Chromium	< 0.05 mg/l	0.05
Cobalt	< 0.05 mg/l	0.05
Copper	< 0.02 mg/l	0.02
Iron	< 0.1 mg/l	0.1
Lead	< 0.05 mg/l	0.05
Magnesium	2.2 mg/l	0.5
Manganese	< 0.01 mg/l	0.01
Molybdenum	< 0.1 mg/l	0.1
Nickel	< 0.04 mg/l	0.04
Potassium	1.0 mg/l	0.5
Silver	< 0.01 mg/l	0.01
Sodium	13.3 mg/l	0.5
Titanium	< 0.05 mg/l	0.05
Vanadium	< 0.05 mg/l	0.05
Zinc	< 0.02 mg/l	0.02
* Analyzed by Lancaster Laboratories, Inc.		

GFT Grab Water Sample*		
Date Collected:6/23/92 Date reported: 7/9/92		
Analysis	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BBC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
* Analyzed by Lancaster Laboratories, Inc.		

APPENDIX 5 - ANALYTICAL METHODOLOGY

SUMMARY

The analytical procedure for crotonaldehyde consisted of derivatization and extraction followed by gas chromatography of the extract. Test and control solutions containing crotonaldehyde were derivatized with 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxamine HCl and sodium thiosulfate. Samples were then extracted once with hexane and an aliquot of the extract was analyzed on a gas chromatograph fitted with an electron capture detector (GC-ECD).

The analytical method was validated twice on separate days using diluent (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$) water samples fortified with crotonaldehyde at a concentration of 20.20 mg/mL. Samples were diluted as necessary prior to derivatization and extraction so that the final concentration in the extract would fall within the range of 1-10 mg/L. Recoveries of crotonaldehyde from the validation test samples averaged 88.5 ± 5.8%, with a limit of quantitation (LOQ) of 2.71×10^{-4} mg/mL. The mean recovery (standard deviation) was used to define limits for acceptance of Quality Control sample performance during ecotoxicology studies performed with crotonaldehyde. This range is established as three standard deviations from the mean recovery obtained during this method validation for crotonaldehyde, and was defined as 71.2 to 106%.

EXPERIMENTAL

Equipment

1.	Instrument:	Hewlett Packard Gas Chromatograph Model 5890 equipped with a Hewlett Packard Model 7673A autosampler, Hewlett Packard Model Ni-63 electron capture detector and Hewlett Packard Model 3396A integrator.
2.	Balance:	SP 182, four place analytical balance, ± 0.1 mg
3.	Laboratory glassware:	syringes, volumetric pipets, volumetric flasks, graduated cylinders, test tubes, GC vials, and amber serum bottles.

Reagents

1. Hexane: reagent grade, Burdick & Jackson
2. Sodium sulfate: anhydrous
3. 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl: Aldrich, 99+%, Lot # 03014MY
4. Sodium thiosulfate: Aldrich, 99+%, Lot # 04901JY

Test Material

Crotonaldehyde, Lot # 7-92, was received from Eastman Kodak Company, Rochester, New York on 23 July 1992 and was identified by the Sponsor to contain 93.8% active ingredient.

Instrumental Conditions

The gas chromatographic analysis was performed utilizing the following instrumental conditions:

Column:	DB-5, 30 m (length) x 0.319 mm I.D.
Gas flows:	Carrier gas - Helium, 3.33 mL/min.
	Make-up gas - Helium, 81.5 mL/min.
Temperatures:	injector - 230 °C
	Column - 100 to 250 °C ramp, 10 °C/minute,
	Detector - 300 °C
Injection Volume:	1 µL
Attenuation:	2^8
Threshold:	9
Peak Width:	0.04 minutes
Retention Time:	crotonaldehyde ≈ 6.8 min.

PROCEDURES

Preparation of Stock Solutions for the Analytical Standards

A new stock solution of crotonaldehyde was prepared on each of the two days the analytical method was validated. Solutions were prepared by weighing 0.1081 g (1st validation) and 0.1083 (2nd validation) of the test material, which corresponded to approximately 0.100 g of active ingredient, into 100-mL volumetric flasks and diluting to volume with NANOpure® water. These stock solutions (1.01 mg/mL and 1.02 mg/mL) were used in the preparation of the analytical standards.

A new solution of the derivatizing reagent, 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxamine HCl, was prepared on each of the two days the analytical method was validated. Solutions were prepared by weighing 0.1015 g (1st validation) and 0.1016 g (2nd validation) of the derivatizing reagent into 100-mL volumetric flasks and diluting with NANOpure® water. The final concentration of the derivatizing reagent was 1.00 mg/mL.

Sample Fortification

Method validation/recovery samples were prepared on two occasions by weighing 2.1582 and 2.1580 g (2.02 gram as active ingredient) into 100 mL volumetric flasks and diluting to volume with ASTM Type II (NANOpure®) water. Triplicate aliquots (0.500 mL) were removed from these primary solutions (20.20 mg/mL) and diluted 4000X with diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$). An additional six diluent water samples were left unfortified and undiluted to be utilized as control samples.

Sampling Techniques

Sampling procedures typically include syphoning (using silicone tubing) from the midpoint of the test container (i.e., glass volumetric flasks, centrifuge tubes, or aquaria) into graduated cylinders for volumes greater than 100 mL, and pipetting (using volumetric pipets) from the midpoint of the test container for sample volumes less than or equal to 100 mL. Deviations from these practices, if any, are identified in the study report.

Derivatization and Extraction

To prepare the control solutions (reagent blanks), 1 mL of 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl was mixing in a test tube with 200 μL of 0.10 M sodium thiosulfate. After mixing, 10 mL of NANOpure® water were added and this mixture was allowed to stand at ambient temperature for 2 hours. In a similar manner, test samples were prepared by mixing 1 mL of 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl in a test tube with 200 μL of 0.10 M sodium thiosulfate. After mixing the derivatizing solution, 10 mL of each fortified sample were added to the derivitization mixture and allowed to stand at ambient temperature for two hours.

All samples (control and fortified) were then extracted by adding 1 - 3 drops of concentrated sulfuric acid to each test tube and mixed. A volume of 2 mL of hexane was added and the contents again shaken for 30 seconds. After allowing the test tube to stand for 15 minutes, the hexane was decanted from the aqueous solution and dried with sodium sulfate to remove any residual water. The sample was then transferred into a GC vial for analysis by gas chromatography (GC) using electron capture detection (ECD).

ANALYSIS

Preparation of Standards

A new set of standard solutions was prepared on each of the two days the analytical method was validated. The concentrations of crotonaldehyde in the standards were 10.1, 5.10, 2.53 and 1.01 mg/L (1st day) and 10.2, 5.10, 2.55, and 1.02 mg/L (2nd day). The standards were derivatized and extracted in the same manner as the samples. Injection of the samples and standards onto the chromatographic system was performed by programmed injection. Two complete sets of standards were analyzed with each sample set, one prior to the samples and one immediately following the samples.

CALCULATIONS

The following equations were used to calculate the measured concentrations of crotonaldehyde:

$$\frac{(\text{signal} - b)}{m} = DC$$

$$DC \times DF = A$$

where:

signal = summation of the two peak signals (heights) from chromatogram

b = y-intercept from regression analysis

m = slope from regression analysis

DC = detected concentration (mg/L) in the extract on GC

DF = dilution factor (final volume of the extract divided by the original aqueous volume extracted)

A = analytical result (mg/L), concentration in the original aqueous sample

The limit of quantitation (LOQ) was calculated using the following equation:

$$\frac{((0.5 \times A_{LS}) - b)}{m} = LOQ_{INST}$$

$$LOQ_{INST} \times DF_{CNTL} = LOQ$$

where:

A_{LS} = The mean signal response of the low concentration standard (two injections)

LOQ_{INST} = The minimum detected level on the instrument (extract)

DF_{CNTL} = The dilution factor of the control samples (smallest dilution factor used) = 1

LOQ = The minimum quantifiable level reported for samples regression analysis or point to point calibration (limit of quantitation)

RESULTS AND DISCUSSION

The mean recovery of crotonaldehyde in diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$) was 88.5 ± 5.8%, for samples with a nominal concentration of

20.20 mg/mL. The limit of quantitation for this method validation was 2.71 x 10^{-4} mg/mL. The LOQ may vary somewhat during subsequent analyses (ecotoxicology testing programs) since it is dependent upon the linear regression of the standards and the peak response (heights) of the low standards. These parameters, while relatively constant, do deviate somewhat and produce small variations in the LOQ. Recovery results from this method validation were used to evaluate Quality Control samples prepared during subsequent ecotoxicology studies involving crotonaldehyde. Quality Control sample recovery expectations were three standard deviations from the mean recoveries obtained in method validation, 71.2 to 106%.

Analytical results for the recovery of crotonaldehyde from diluent water are presented in Table 1A. A representative chromatogram showing the analysis of derivatized crotonaldehyde in one of the standards is shown in Figure 1A. A representative chromatogram showing the analysis of derivatized crotonaldehyde from one of the fortified diluent water samples is shown in Figure 2A. The analysis of control water is presented in Figure 3A. A typical linear regression analysis for derivatized crotonaldehyde is presented in Figure 4A.

Table 1A. Analytical results for the recovery of crotonaldehyde from diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$).

Fortified Concentration (mg/mL)	Volume Extracted (mL)	Recovered Concentration (mg/mL)	Percent Recovery[a] (%)
20.20	10.0	17.47	86.5
20.20	10.0	19.61	97.1
20.20	10.0	17.61	87.2
20.20	10.0	16.45	81.4
20.20	10.0	18.24	90.3
20.20	10.0	26.39	130.6[b]
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA

NA = Not Applicable

Mean recovery: 88.5 ± 5.8%, (N = 5).

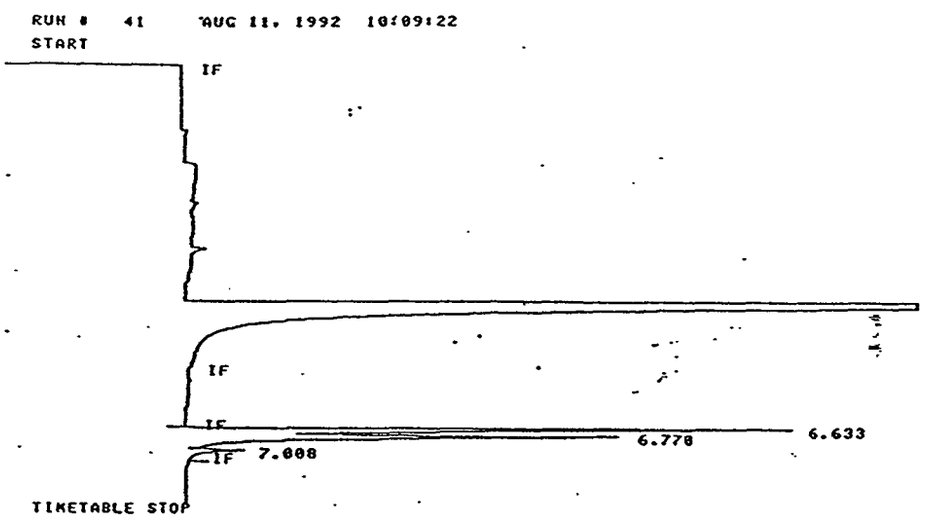
Limit of quantitation has been determined to be 2.71×10^{-4} mg/mL.

Values expressed as less than are below the limit of quantitation (LOQ). The LOQ for each sample is dependent upon the sample volume, dilution factor, and standard concentration range.

[a] Values presented are based on unrounded analytical results rather than the rounded values presented in this table.

[b] High percent recovery was determined to be an outlier using Chauvenet's Criterion and was not included in the calculation of the mean recovery.

Figure 1A. Chromatogram of derivatized crotonaldehyde from one of the standards.



RUN# 41 AUG 11, 1992 10:09:22

SAMPLE NAME: STD SAMPLE# 5
10 NG/L

IDENTIFIER : 2728A12338

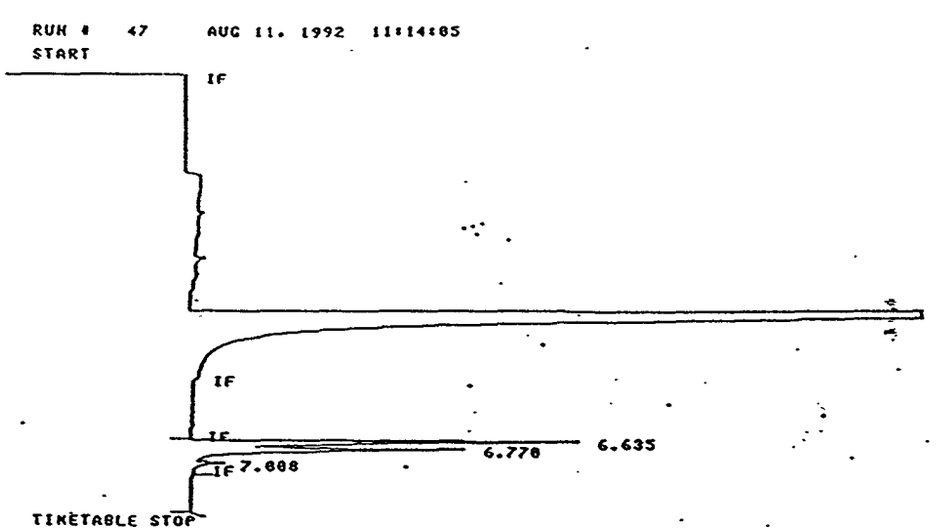
CROT

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	NG/L	NAME
6.800	++	10806808	.066	2732466	1R	5.503	CROT

TOTAL AREA=1.0807E+07
MUL FACTOR=1.0000E+00

Figure 2A. Chromatogram showing derivatized crotonaldehyde recoveries from one of the fortified samples.



RUN# 47 AUG 11, 1992 11:14:05

SAMPLE NAME: SK-C SAMPLE# 11

(V) ~~20000~~ NG/L
20260

IDENTIFIER : 2728A12358

CROT

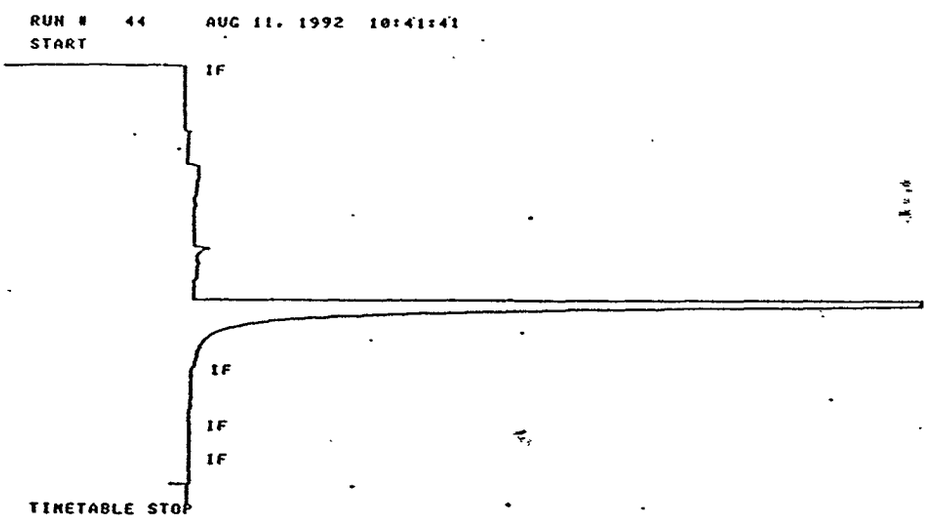
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	NG/L	NAME
6.808	**	6779206	.865	1742420	IR	16136.492	CROT

TOTAL AREA=6779206
MUL FACTOR=5.0000E+03

(P) Data replaced upon changing the S.I. of test material
10/20/92 KT

Figure 3A. Chromatogram showing analysis of one of the control water samples.



RUN# 44 AUG 11, 1992 10:41:41

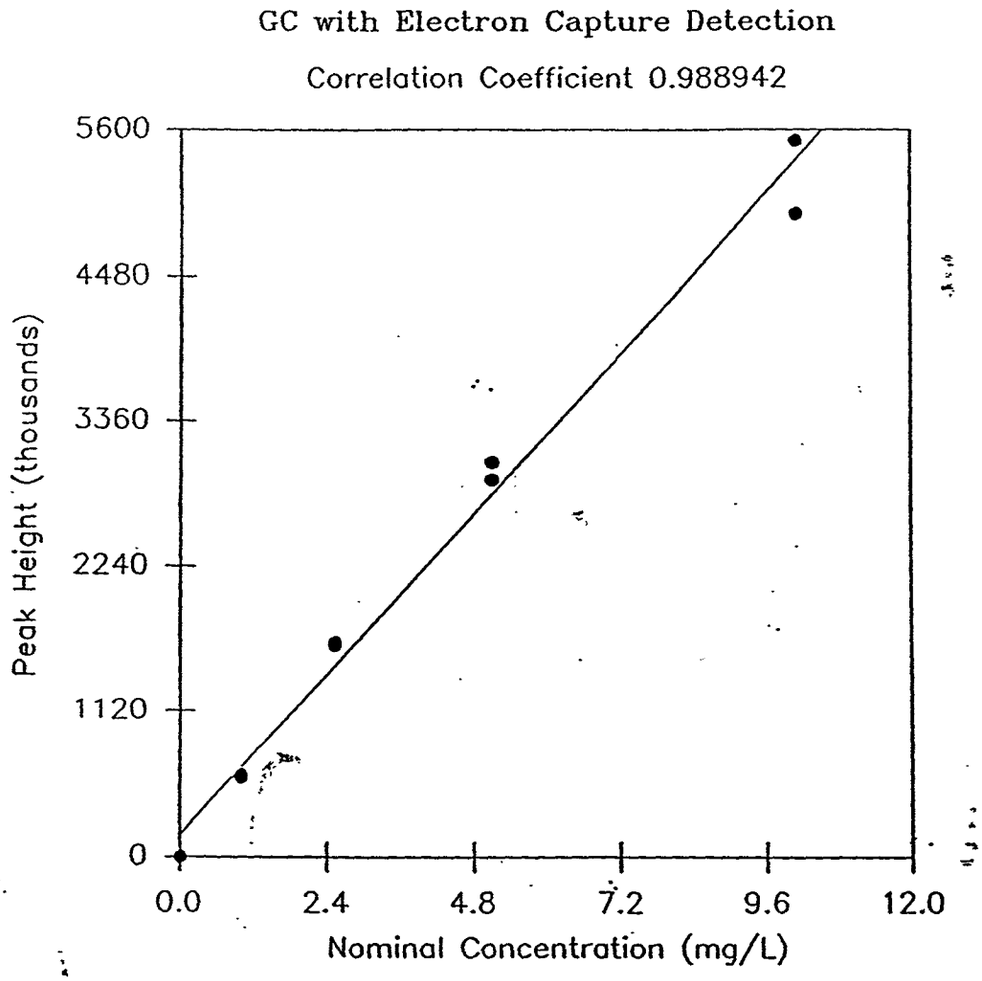
SAMPLE NAME: CONT C SAMPLE# 8
0.00 MG/L

IDENTIFIER : 2728A12358

CROT

NO RUN PEAKS STORED

Figure 4A. **Plot of signal response versus concentration for derivatized crotonaldehyde linear regression analysis.**



APPENDIX 6 - CHEMICAL DISTRIBUTION RECORD

1852 0692 6103 130

(220)

page 36
SLI# 28-36

Test Material Log + Usage Book

Test Material: CROTONALDEHYDE Synonym
Received from: EASTMAN KODAK CO City/State Rochester, NY 14650
Sponsor: EASTMAN KODACK City/State
Telephone #
Date received JULY 23, 1992 Date logged: JULY 23, 1992

Label information only:

Test Material CROTONALDEHYDE Net Wt. na
(Lot,) Batch, Code, I.D. Other # T-92 Purity: na
Expiration Date na
Other Information: Storage: Under Nitrogen Tare Wt: 466.4 TOTAL WT: 1303.9g

Sponsor Information: Source by on
Test Material
Lot, Batch, Code, I.D. Other#: Purity:
as Salt as Base
CAS # [ ]
Molecular Wt: g/mole Solubility: (units)
Empirical Formula: Vapor Pressure:
Storage Conditions: Under nitrogen - THC Refrig Dissociation Constant(s):
Other: NET WT.: ONE LITER

Radiolabelled:(only) Source na by na on na
Amount (mCi) na Sp.Activity na (units)
Radiochemical Purity: na Salt na Base na
Other

Characterization: By Date
Color:
Solid Liquid Gas
Powder Viscous
Crystal
Pellet Other

(271)

Gross Wt. 1306.54g
Storage location:_____ Container: Amber Bottle

Hazard Rating: 3 by JMG on 6-18-92

Shipping Info:
Hazardous ✓ Non-hazardous ng
Classification: Flammable Liquid / Poison By Inhalation
DOT Label: Flammable Liquid + Poison
UN# 1143
Contact: Joe Gorsuch telephone # 716-588-2140

transcribed by pl on 7-23-92
verified by _____ on _____

Disposition of test material:
Returned to _____ by _____ on _____
Final Weight: _____

Report No. 92-10-4473

SPRINGBORN LABORATORIES, INC.

(A) This total was comprised of entries made 7/14/92 thru 7/24/92. These were recorded on the wrong SLI #. The total of 7.9375 will be deducted from the total used as of 9/28/92.

28-36

MOVED TO HSR

Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used	By	Study Number
7-28-92	1306.72			MS	1.3978	1.3978	NSP	1852.6103.130
7-28-92					0.1014	1.4992	JV	1852 6103-130
8-3-92					0.1015	1.6007	JV	1852 6103-250
8-4-92					0.1085	1.7092 (1) ~~2.6857~~	JV	1852 6103
8-7-92					0.1082	1.8174 ~~2.7939~~	JV	1852-6103-250
8-7-92					2.1576	3.9750 ~~4.7815~~	JV	1852-6103-250
8-7-92					0.1081	5.0831 ~~5.8896~~	JV	1852-6103-250
8-7-92					2.1580	6.2411 ~~7.2476~~	JV	1852-6103-250
8-10-92					0.1083	6.3494 ~~7.3559~~	JV	1852-6103-250
8-10-92					2.1582	8.5076 ~~9.5141~~	JV	1852-6103-250
8/17/92					5.5022	14.0098 ~~15.0163~~	MB	1852-6103.130
8-17-92					2.1579	16.1677 ~~17.1742~~	JV	1852 6103-130
8-17-92					0.1080	16.2757 ~~17.2822~~	JV	1852 6103-130
8-18-92					0.1080	16.3837 ~~17.3902~~	JV	185-26103-130
8-18-92					2.1581	18.5418 ~~19.5483~~	JV	185-26103-130
8-19-92					2.1585	20.7003 ~~21.7068~~	JV	185-26103-130
8.20.92					2.2650	22.9653 ~~23.9718~~	RR	1852.6103
* 7/14/92					0.9923	23.9576 ~~24.9641~~	LCP	1852.6103.130
* 7/16/92					0.9921	24.9497 ~~25.9562~~		
* 7/18/92					0.9924	25.9421 ~~26.9486~~		
* 7/18/92					0.9924	26.9345 ~~27.9410~~		
* 7/20/92					0.9922	27.9267 ~~28.9332~~		
* 7/22/92					0.9924	28.9188 ~~29.9253~~		
* 7/24/92					0.9921	29.9109 ~~30.9174~~		
* 7/24/92					0.9919	30.9028 ~~31.9093~~		
* 7/28/92					4.9599	35.8627 ~~36.8692~~		

Pg Sub 11-4-92

(N) Total Used: 7.9375

* (LE) HCL 8/25/92

(1) Error in calculation MS 9-21-92

SPRINGBORN LABORATORIES, INC.

	Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used	By	Study Number
*	7/30/92					0.9924	36.8551 ~~37.8607~~ ①	PER	1852.0692.6102-110
*	8/4/92					~~3.5225~~ 3.5635	40.4186 ~~41.4251~~ ②		
*	8/5/92					3.5620	43.9806 ~~44.9871~~ ③		
*	8/6/92					3.5628	47.5434 ~~48.5499~~ ④		
*	8/7/92					3.5620	51.1054 ~~52.1119~~ ⑤		
*	8/8/92					3.5624	54.6678 ~~55.6743~~ ⑥		
*	8/9/92					3.5625	58.2303 ~~59.2368~~ ⑦		
*	8/10/92					3.5608	61.7911 ~~62.7976~~ ⑧		
ⓦ	8/20/92					3.6577	65.4488 ~~66.4477~~ ⑨	MOB	1852.0692.6103.130
ⓦ	8/22/92					3.6513	69.094 ~~70.1001~~ ⑩	MOB	1852.0692.6103.138
ⓦ	8/24/92					3.6515	72.7455 ~~73.7516~~ ⑪	MOB	1852.0692.6103.130
	8/24/92					3.6523	76.3979 ~~77.4044~~ ⑫	MOB	1852.0692.6103.138
	8-26-92					2.1584	78.5563 ~~79.5628~~ ⑬	JV	1852-0692-6103
	8-26-92					0.1080	78.6643 ~~79.6708~~ ⑭	JV	1852-0692-6103
*	8-25-92					01083	78.7726 ~~79.7771~~ ⑮	JV	
*	8-25-92					2.1579	80.9305 ~~81.9371~~ ⑯		
*	8-24-92					0.1083	81.0388 ~~82.0453~~ ⑰		
*	8-24-92					2.1583	83.1971 ~~84.2036~~ ⑱		
	8-28-92					3.6522	86.8493 ~~87.8558~~ ⑲	MOB	1852.0692.6103.130
	8-28-92					0.1083	86.9576 ~~87.9641~~ ⑳	JV	1852-0692-6103
	8-31-92					0.1083	87.0659 ~~88.0724~~ ㉑		
	8-31-92					2.1582	89.2241 ~~90.2306~~ ㉒		
	9-5-92					3.6520g	93.8761 ~~93.8826g~~ ㉓	BAT	1852.0692.6103.130
	9-7-92					9.131g	103.0074 ~~103.0139g~~ ㉔	BAT	1852.0692.6103.130
	9-7-92					0.1083	102.1154 ~~103.1219~~ ㉕	JV	1852-0692-6103
	9-7-92					2.1581	104.2735 ~~105.280~~ ㉖	JV	1852-0692-6103

Delete 8/26/92 ③ Error in Calculation ie KS 9-21-92

* Deleted 8/25/92 (...)

Report No. 92-10-4473

SPRINGBORN LABORATORIES, INC.

SLI#

① Calculation Error MS 9-21-92

Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used ①	By	Study Number
9-14-92				JV	0.1083	~~104.3818~~ ~~105.3883~~	JV	1852-6102-
9-14-92					2.1579	~~106.5397~~ ~~107.5462~~ ①	JV	1852-6102
9-14-92					9.1310	~~115.6707~~ ~~116.6772~~ ①	MDB	1852.0692.6103.130
9-3-92					3.6523	~~119.3230~~ ~~120.3295~~ ①	MDB	1852.0692.6103.130
9/21/92					9.1309	128.4539	MDB	1852.0692.6103.BG
8-21-92					0.1081	128.562	JV	1852-0692-6103
8-21-92					2.1584	130.7204	JV	1852-0692-6103
9-21-92					0.1079	130.8283	JV	1852-0692-6103
9-21-92					2.1579	132.9862	JV	1852-0692-6103
② 8/30/92					3.6521	136.6383	WC	1852.0692.6102.1
② 9/1/92					3.6520	140.2903	WC	1852.0692.6102.1
9-28-92					0.1081	140.3984	JV	1852-0692-6103
9-28-92					2.1579	142.5563	JV	1852-0692-6103
10-5-92					2.1579	144.7142	JV	1852-0692-6102
10-5-92					0.1081	144.8223	JV	1852-0692-6103
9/28/92					9.1311	④ ~~143.9559~~ 146.0159	MDB	1852.0692.6103.130

Bkg sub 11.23.92

Bkg sub 11.23.92

APPENDIX 7 - STATISTICAL ANALYSES

MATC Program Methods and Calculations

1. **Dunnett's Test** is a parametric procedure which assumes normal distribution and homoscedasticity, and compares each of the group means to the control mean and determines if the two are significantly different. This procedure is used as a one-sided test to test at a 95% level of significance. Equal sample sizes are desirable, however, TOXSTAT[1] can adequately deal with unequal sample sizes by calculating a critical value for each comparison.

2. **Williams' Test** is a parametric procedure considered to be preferable for chronic toxicity testing, but by design, assumes that the mean response of a variate is a monotonic function of concentration. Similar to Dunnett's Test, the Williams' test compares each of the group means to the control. However, it is used in a "step-down" manner (according to treatment levels) which enables the analysts to determine the concentration at which the monotonic function deteriorates, hence evidence for a significant response.

3. **Kruskal-Wallis Test** is an analogous nonparametric procedure that is used when data are not normally distributed or when group variances are not homogeneous. The null hypothesis for this test is not based on the metric of a specific parameter, but rather on the magnitude of the difference in rank distribution of the variates (this procedure ranks the variates).

[1]Gulley, David. D., Ann M. Boelter and Harold L. Bergman. 1988. TOXSTAT, Release 2.1, University of Wyoming, Laramie, Wyoming.

Representative Statistical Output

(205B)

ptonaldehyde Chronic 1852.0692.6103.130
le: a:SURVIVAL. Transform: NO TRANSFORMATION

ppiro Wilks test for normality

D = 0.068

= 0.860

itical W (P = 0.05) (n = 24) = 0.916
itical W (P = 0.01) (n = 24) = 0.884

ta FAIL normality test. Try another transformation. ①

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

① When variance was added to 3 concentrations with 100% survival (15,0.38,0.094 mg/L), the data set passes Shapiro Wilks. See pg 204. MSP 10/2/92

203C

Crotonaldehyde Chronic 1852.0692.6103.130
le: a:SURVIVAL. Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 135.19
Table Chi-square value = 15.09 (alpha = 0.01)
ble Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
ed for Chi-square table value ==> df (#groups-1) = 5

---

ta FAIL homogeneity test at 0.01 level. Try another transformation. ①

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

① When variance is added to the 3 concentrations with 100% survival (1.5, 0.38, 0.094 mg/L), Then Bartletts test passes. See pg 205. AEP 10/6/92

(204)

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:\survival Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

W = 0.199

= 0.947

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

205

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:\survival Transform: ARC SINE(SQUARE ROOT(Y))

Bartletts test for homogeneity of variance

Calculated B statistic = 4.04
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

306

TITLE: Crotonaldehyde Chronic 1852.0692.6103.130
FILE: a:\survival
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.4120
1	Control	2	0.9000	1.2490
1	Control	3	1.0000	1.4120
1	Control	4	1.0000	1.4120
2	0.094	1	1.0000	1.4120
2	0.094	2	1.0000	1.4120
2	0.094	3	1.0000	1.4120
2	0.094	4	1.0000	1.4120
3	0.19	1	1.0000	1.4120
3	0.19	2	0.9000	1.2490
3	0.19	3	0.8000	1.1071
3	0.19	4	0.7000	0.9912
4	0.38	1	1.0000	1.4120
4	0.38	2	1.0000	1.4120
4	0.38	3	1.0000	1.4120
4	0.38	4	1.0000	1.4120
5	0.75	1	1.0000	1.4120
5	0.75	2	0.9000	1.2490
5	0.75	3	0.9000	1.2490
5	0.75	4	1.0000	1.4120
6	1.5	1	1.0000	1.4120
6	1.5	2	1.0000	1.4120
6	1.5	3	1.0000	1.4120
6	1.5	4	1.0000	1.4120

(205)

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:\survival Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	4	1.249	1.412	1.371
2	0.094	4	1.412	1.412	1.412
3	0.19	4	0.991	1.412	1.190
4	0.38	4	1.412	1.412	1.412
5	0.75	4	1.249	1.412	1.331
6	1.5	4	1.412	1.412	1.412

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:\survival Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.007	0.081	0.041
2	0.094	0.000	0.000	0.000
3	0.19	0.033	0.182	0.091
4	0.38	0.000	0.000	0.000
5	0.75	0.009	0.094	0.047
6	1.5	0.000	0.000	0.000

(208)

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:\survival Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.152	0.030	3.750
Within (Error)	18	0.146	0.008	
Total	23	0.297		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

(209)

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:SURVIVAL. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	0.975	0.975	0.942
2	0.094	4	1.000	1.000	0.942
3	0.19	4	0.850	0.850	0.942
4	0.38	4	1.000	1.000	0.975
5	0.75	4	0.950	0.950	0.975
6	1.5	4	1.000	1.000	1.000

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:SURVIVAL. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.942				
0.094	0.942	0.770		1.73	k= 1, v=18
0.19	0.942	0.770		1.82	k= 2, v=18
0.38	0.975	0.000		1.85	k= 3, v=18
0.75	0.975	0.000		1.86	k= 4, v=18
1.5	1.000	0.577		1.87	k= 5, v=18

s = 0.061
Note: df used for table values are approximate when v > 20.

2094

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:SURVIVAL. Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.069	0.014	3.500
Within (Error)	18	0.068	0.004	
Total	23	0.136		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:SURVIVAL. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.975	0.975		
2	0.094	1.000	1.000	-0.559	
3	0.19	0.850	0.850	2.795	*
4	0.38	1.000	1.000	-0.559	
5	0.75	0.950	0.950	0.559	
6	1.5	1.000	1.000	-0.558	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

Crotonaldehyde Chronic 1852.0692.6103.130
File: a:SURVIVAL. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	4			
2	0.094	4	0.108	11.1	-0.025
3	0.19	4	0.108	11.1	0.125
4	0.38	4	0.108	11.1	-0.025
5	0.75	4	0.108	11.1	0.025
6	1.5	4	0.108	11.1	-0.025

Crotonaldehyde Chronic 1852.0692.6103.130

APPENDIX 8 - EXCERPTED RAW DATA

SPRINGBORN LABORATORIES, INC. Page 18

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK COMPANY
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6103-130 (1852-0692 1102-124)
Test Type: 21 DAY LIFE CYCLE W/DH
Sample Date(s): PRE-TEST II (AUG-21-92)
Data Entered By: RT RT
Date Program Run: 22-Aug-92

Minimum Detection Limits:
Peak HEIGHT = 480933.000000
Y-evaluate = 0.211994 mg/L

Sample ID	Nominal Concentration (mg/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (mg/L)	Analytical Result (mg/L)	Percent of Nominal
8-92-1073	17000	6.8	2978838	4000	4.002E+00	1.601E+04	94.2
8-92-1074	17000	6.8	3049744	4000	4.109E+00	1.644E+04	96.7
8-92-1075	17000	6.8	3221445 ~~3221445~~ ⊙	4000	4.370E+00 ~~4.370E+00~~	1.748E+04 ~~1.748E+04~~ ⊙	103 ~~103~~ ⊙ 108
8-92-1076 RB	0	NO PEAK	< 480933	4000	< 0.2120	< 847.9756	NA
8-92-1077QA1	20000	6.8	3495379	4000	4.786E+00	1.914E+04	95.7
8-92-1078QA2	20000	6.8	3951477	4000	5.478E+00	2.191E+04	110 **
8-92-1079QA3	20000	6.8	3983634	4000	5.526E+00	2.211E+04	111 **
8-92-1080-96	17000	6.8	3570472	4000	4.899E+00	1.960E+04	115 *
8-92-1081-96	17000	6.8	3066203	4000	4.134E+00	1.654E+04	97.3 *

* Stock stability sampling at 96 Hrs in/none thru. 8/24/92 RT
** QA beyond accepte std. dev.

Note: Data has been re-processed due to changing the M.W. of test material. (See p. 19) and p. 5 of 5B 10/20/92 RT

⊙ TE JDS 10-30-92

SPRINGBORN LABORATORIES, INC. Page 30

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK COMPANY
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6103-130 (1852-0692-6102-120)
Test Type: 21 DAY LIFE CYCLE W/DM
Sample Date(s): DAY 0 & PRE-TEST 1 ELS W/FHM
Data Entered By: RT
Date Program Run: 26-Aug-92

Minimum Detection Limits:
Peak HEIGHT = 733612.750000
Y-evaluate = 0.359101 mg/L

Sample ID	Nominal Concentration (mg/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (mg/L)	Analytical Result (mg/L)	Percent of Nominal
8-92-1169	17000	6.6	4986682	4000	5.702E+00	2.281E+04	134
8-92-1170	17000	6.6	4600794	4000	5.217E+00	2.087E+04	123
8-92-1168	17000	6.6	1891224	4000	1.813E+00	7.254E+03	42.7 *
8-92-1172QA1	20000	6.6	3641422	4000	4.012E+00	1.605E+04	80.2
8-92-1173QA2	20000	6.6	4187848	4000	4.699E+00	1.879E+04	94.0
8-92-1174QA3	20000	6.6	4405651	4000	4.972E+00	1.989E+04	99.4
8-92-1176	17000	6.6	4723910	4000	5.372E+00	2.149E+04	126
8-92-1178	17000	6.6	4745456	4000	5.399E+00	2.160E+04	127
8-92-1171	0	NO PEAK	< 733613	1	< 0.3591	< 0.3591	NA

* Data not representative of exposure solutions will not be included in the study. 8/26/92 RT

Note: Data has been re-processed due to the changing of the A.I. of test material. (see p. 58 and p. 31) JGR 10/21/92
10/20/92 RT

Report No. 92-10-4473

SPRINGBORN LABORATORIES, INC. Page 42

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6102-120 (1852-0692-403-130)
Test Type: ELS W/FHM
Sample Date(s): PRE-TEST II (AUG-25-92)
Data Entered By: RT
Date Program Run: ~~31-Aug-92~~ 25-Aug-92

Minimum Detection Limits:
Peak HEIGHT = 818058.750000
Y-evaluate = 0.561310 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
8-92-1221	17000	6.6	5440634	4000	5.740E+00	2.296E+04	135
8-92-1222	17000	6.6	5485603	4000	5.791E+00	2.316E+04	136
8-92-1223	0	NO PEAK	< 818059	1	< 0.5613	< 0.5613	NA
8-92-1224QA1	20000	6.6	5092224	4000	5.350E+00	2.140E+04	107 *
8-92-1225QA2	20000	6.6	4902528	4000	5.137E+00	2.055E+04	103
8-92-1226QA3	20000	6.6	4351718	4000	4.520E+00	1.808E+04	90.4
8-92-1220	17000	6.6	5470506	4000	5.774E+00	2.310E+04	136

* QA beyond acceptable std. dev. JBS 8-21-92

Ø IE RT 8/25/92

Note: Data has been re-processed due to changing the A.I. of test material. (See p. 5-58 & 43)
10/20/92 RT

SPRINGBORN LABORATORIES, INC. Page 54

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6102-120 (1857-0692 -6103-130)
Test Type: ELS W/FHM
Sample Date(s): DAY 0 (AUG-26-92)
Data Entered By: RT RT
Date Program Run: 29-Aug-92

Minimum Detection Limits:
Peak HEIGHT = 916020.750000
Y-evaluate = 0.581241 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
8-92-1227	17000	6.6	4970592	4000	5.256E+00	2.102E+04	124
8-92-1228	17000	6.6	5103402	4000	5.409E+00	2.164E+04	127
8-92-1229	17000	6.6	4667574	4000	4.907E+00	1.963E+04	115
8-92-1231QA1	20000	6.6	5562522	4000	5.939E+00	2.376E+04	119 *
8-92-1232QA2	20000	6.6	5407322	4000	5.760E+00	2.304E+04	115 *
8-92-1233QA3	20000	6.6	6109328	4000	6.569E+00	2.628E+04	131 *
8-92-1230 RB	0	NO PEAK	< 916021	1	< 0.5812	< 0.5812	NA
8-92-1234	17000	6.6	6199066	4000	6.673E+00	2.669E+04	157
8-92-1235	17000	6.6	6302630	4000	6.792E+00	2.717E+04	160

QA beyond acceptable std dev. range SBS 9-21-92

Note: Data has been re-processed due to changing the A.I. of test material. (see p. 5-58 and 55)

SPRINGBORN LABORATORIES, INC. Page 66

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6103-130 (1852-0692-6102-120)
Test Type: D.MAGNA CHRONIC,AND FHM ELS
Sample Date(s): DAY 7/5 (AUG-31-92)
Data Entered By: JV RT
Date Program Run: 01-Sep-92

Minimum Detection Limits:
Peak HEIGHT = 754076.250000
Y-evaluate = 0.210047 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
8-92-1411	17000	6.6	5001242	4000	5.036E+00	2.015E+04	119
8-92-1412	17000	6.6	4981024	4000	5.013E+00	2.005E+04	118
8-92-1413	17000	6.6	5053392	4000	5.096E+00	2.038E+04	120
8-92-1414RB	0	NO PEAK	< 754076	1	< 0.2100	< 0.2100	NA
8-92-1415QA1	20000	6.6	5839235	4000	5.989E+00	2.395E+04	120 *
8-92-1416QA2	20000	6.6	5842848	4000	5.993E+00	2.397E+04	120 *
8-92-1417QA3	20000	6.6	5808774	4000	5.954E+00	2.382E+04	119 *
8-92-1418	17000	6.6	4334019	4000	4.278E+00	1.711E+04	101
8-92-1419	17000	6.6	4491770	4000	4.457E+00	1.783E+04	105

* Beyond acceptable std dev. range.. RT 9-24-92

Note: Data has been re-processed due to changing the A.I. of test material. (see p. 55 & 67) 10/20/92 RT

SPRINGBORN LABORATORIES, INC. Page 90

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE (1852-0692 -6103-150)
Project No.: 1852-0692-6102-120
Test Type: DAPHNIA MAGNA CHRONIC, AND FHM ELS
Sample Date(s): DAY 14,AND FHM ELS DAY 5 (09-07-92)
Data Entered By: JV
Date Program Run: 08-Sep-92

Minimum Detection Limits:
Peak HEIGHT = 753203.250000
Y-evaluate = 0.396738 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
9-92-310	17000	6.6	6003446	4000	5.600E+00	2.240E+04	132
9-92-311	17000	6.6	5648282	4000	5.248E+00	2.099E+04	123
9-92-312	17000	6.6	5905114	4000	5.503E+00	2.201E+04	129
9-92-313RB	0	NO PEAK	< 753203	1	< 0.3967	< 0.3967	NA
9-92-314QA1	20000	6.6	4950234	4000	4.556E+00	1.823E+04	91.1
9-92-315QA2	20000	6.6	4928282	4000	4.535E+00	1.814E+04	90.7
9-92-316QA3	20000	6.6	5014810	4000	4.620E+00	1.848E+04	92.4
9-92-317	17000	6.6	5707981	4000	5.307E+00	2.123E+04	125
9-92-318	17000	6.6	5895082	4000	5.493E+00	2.197E+04	129

Note: Data has been re-processed due to changing the A.I. of test material. (See p. 5-58 & 91)
10/20/92 NT

SPRINGBORN LABORATORIES, INC. Page 102

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6102-120 (1852-6082 / 6103-130)
Test Type: ELS W/FHM, AND 21 DAY LIFE CYCLE W/DM
Sample Date(s): DAY 12(ELS/FHM) DAY 21(DM) (09-14-92)
Data Entered By: JV
Date Program Run: 15-Sep-92

Minimum Detection Limits:
Peak HEIGHT = 995228.250000
Y-evaluate = 0.279029 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
9-92-857	17000	6.6	6435859	4000	4.913E+00	1.965E+04	116
9-92-858	17000	6.6	6040438	4000	4.576E+00	1.830E+04	108
9-92-859	17000	6.6	6481197	4000	4.952E+00	1.981E+04	117
9-92-860 RB	0	NO PEAK	< 995228	1	< 0.2790	< 0.2790	NA
9-92-861QA1	20000	6.6	6855350	4000	5.270E+00	2.108E+04	105
9-92-862QA2	20000	6.6	7057744	4000	5.443E+00	2.177E+04	109 X
9-92-863QA3	20000	6.6	6995142	4000	5.389E+00	2.156E+04	108 X
9-92-864	17000	6.6	6976720	4000	5.374E+00	2.149E+04	126
9-92-865	17000	6.6	6839718	4000	5.257E+00	2.103E+04	124

X QA beyond third std. dev. KT 9/21/92

Note: Data has been re-processed due to changing the S.F. of test material. (See p. 5-38 & 103)
10/21/92 KT

SPRINGBORN LABORATORIES, INC. Page 114

RESULTS OF CHROMATOGRAPHIC ANALYSIS

Sponsor: EASTMAN KODAK
Test Material: CROTONALDEHYDE (1852-0652 -6102-124)
Project No.: 1852-0692-6103-130
Test Type: 21 DAY LIFE CYCLE W/DM, AND ELS W/FHM
Sample Date(s): DAY 28 W/DM, DAY 19 ELS W/FHM (09-21-92)
Data Entered By: JV RT
Date Program Run: 22-Sep-92

Minimum Detection Limits:
Peak HEIGHT = 1425483.500000
Y-evaluate = 0.497791 MG/L

Sample ID	Nominal Concentration (MG/L)	Retention Time (minutes)	Peak HEIGHT (COUNTS)	Dilution Factor	Y Evaluate (MG/L)	Analytical Result (MG/L)	Percent of Nominal
9-92-1379	17000	6.6	7777984	4000	3.748E+00	1.499E+04	88.2
9-92-1380	17000	6.6	7316298	4000	3.512E+00	1.405E+04	82.6
9-92-1381	17000	6.6	7595504	4000	3.655E+00	1.462E+04	86.0
9-92-1382 RB	0	NO PEAK	< 142548	1	< 0.4978	< 0.4978	NA
9-92-1386	17000	6.6	7100490	4000	3.402E+00	1.361E+04	80.0
9-92-1387	17000	6.6	7358349	4000	3.534E+00	1.413E+04	83.1
9-92-1383QA1	20000	6.6	9195059	4000	4.474E+00	1.789E+04	89.5
9-92-1388	17000	6.6	6432298	4000	3.060E+00	1.224E+04	72.0
9-92-1389	17000	6.6	6410307	4000	3.049E+00	1.219E+04	71.7
9-92-1390	17000	6.6	6224547	4000	2.954E+00	1.181E+04	69.5
9-92-1391	17000	6.6	7163354	4000	3.434E+00	1.374E+04	80.8
9-92-1384QA2	20000	6.6	8524595	4000	4.130E+00	1.652E+04	82.6
9-92-1392	17000	6.6	6870938	4000	3.284E+00	1.314E+04	77.3
9-92-1393	17000	6.6	7382589	4000	3.546E+00	1.418E+04	83.4
9-92-1394	17000	6.6	7385053	4000	3.547E+00	1.419E+04	83.5
9-92-1395	17000	6.6	6061786	4000	2.870E+00	1.148E+04	67.5
9-92-1396	17000	6.6	5888189	4000	2.781E+00	1.113E+04	65.4
9-92-1385QA3	20000	6.6	9396262	4000	4.577E+00	1.831E+04	91.5

Note: Data has been re-processed due to changing the A.I. of test material. (See p. 5 & 115) RT 10/21/92

Study #: 1852.0492.6103.130 (6700-120)

Test Material: Crotonaldehyde [redacted] (3)

Page #: 21

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 21-Day Life-Cycle Test Day: Pretest #2 Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☒ (3) Other Nanopure (a)

Sampling Date: 8/21/92 Time: 1100 Sampling Source: Syringe

Extraction/Dilution Date: 8-21-92 Initials: JL Authorization to Sample: ACP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
8-92-1073	17 ①	0.0500	2.00	200	1ml	1ml	4000
1074	17 ①						
1075	17 ①						
1076	Reagent Blank						
1077	QC #1						
1078	QC #2						
1079	QC #3						
1080	17 ②						
1081	17 ②	↓	↓	↓	↓	↓	↓

8/22/92 ~

Comments: ① Pretest #2 - 24 hour old stock samples (prepared 8/20/92). ACP 8/20/92

② Stock stability sample - 96-hour old stock (prepared 8/17/92). ACP 8/20/92

③ IC PT 8-21-92

SPRINGBORN LABORATORIES, INC

0692-

Study #: 1852.6103.130 (1852-0052-6102-120) | Test Material: Carbon Tetrachloride | Page #: 33

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 21 Day Chronic with Daphnia Fifth Early Life Stage | Test Day: Day 0 Day 2nd Fifth Fish ELS | Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Nanopure

Sampling Date: 8/24/92 Time: 1100 Sampling Source: Syphon

Extraction/Dilution Date: 8 24-92 Initials: JV Authorization to Sample: ASP

Sample Identification Number	Nominal Conc. (mg/ml)	1° Sample Vol/Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
S-92-1168	17	0.0580	2.0	200	14	14	4000
1169	17	↓	↓	↓	↓	↓	↓
1170	17						
1171	Reagent Blank						
1172	QC 1						
1173	QC 2						
1174	QC 3	↓	↓	↓	↓	↓	↓

8/24/92

Comments:

MM

SPRINGBORN LABORATORIES, INC

Study #: 1852.0692.6102-120 .6103.130 | Test Material: Crotonaldehyde | Page #: 334

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 21-Day Life-Cycle w/ D. magna (EASD 8/24/92) Fifth Early Life Stage | Test Day: Day 0 D. magna Fresh/1st Fifth Ests | Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ | Soft Reconstituted Water ☐ | Unfiltered Seawater ☐ | Filtered Seawater ☐ | AAP ☑ | Other Nanopure

Sampling Date: 8/24/92 | Time: 1100 | Sampling Source: Syringe

Extraction/Dilution Date: 8-24-92 | Initials: SV | Authorization to Sample: ASP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol/Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
8-92-1176	17①	0.0500	2.00	200	NA	NA	4000
8-92-1178	17①	↓	↓	↓	↓	↓	↓
							NA
			8/24/92				

Comments: Stock prepared (8/17/92). Stock is 7 Days old. AEP 8/24/92

JE 8/27 8-24-92

SPRINGBORN LABORATORIES, INC

Study #: 1852.0692 6103-130 6102.120 | Test Material: Glutaraldehyde | Page #: 45

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 2 Wking Life Cycle w/Rpm Early Life Stage with FHM | Test Day: Day 1 Pulse #2 | Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Diluent

Sampling Date: 8/25/92 Time: 1000 Sampling Source: Aquarium

Extraction/Dilution Date: 8-25-92 Initials: EC Authorization to Sample: RDP

Sample Identification Number	Nominal Conc. (mg/L)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
8-92-1220	17	0.0500	2.00	200	1	1	4000
1221	17	↓	↓	↓	↓	↓	↓
1222	17						
1223	Reagent Blank						
1224	QC						
1225	QC						
1226	QC	↓	↓	↓	↓	↓	↓

8/25/92

Comments:
1225

SPRINGBORN LABORATORIES, INC

Study #: 1852.0692.6102.120 (6103-130) | Test Material: Crotonaldehyde | Page #: 57

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

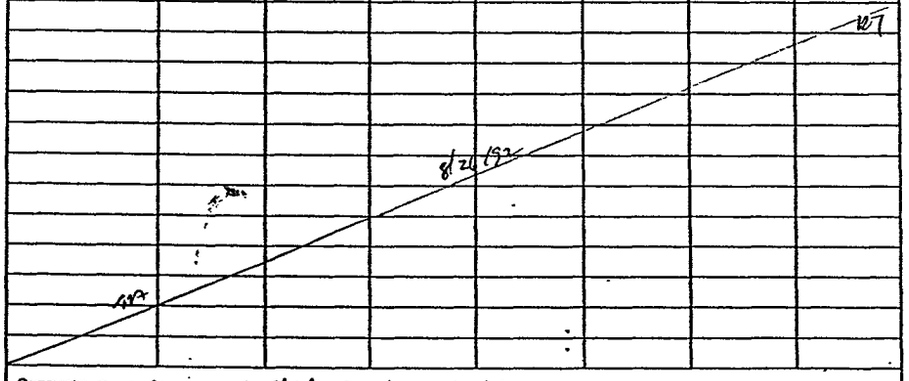
Test Type: 21 Day Life cycle w/mm FHM ELS | Test Day: 8-19-92 0 | Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Nanopure

Sampling Date: 8/20/92 Time: 1000 Sampling Source: Syringe

Extraction/Dilution Date: 8-26-92 Initials: JC Authorization to Sample: ADP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/Vi)
B-92-1227	17①	0.0500	2.00	200	NR	NR	4000
1228	17①	↓	↓	↓	↓	↓	↓
1229	17①						
1230	Reagent Blank						
1231	QC#1						
1232	QC#2						
1233	QC#3						
1234	17②						
1235	17②	↓	↓	↓	↓	↓	↓



Comments: ① Stock prepared 8/20/92 in Nanopure water
② Stock prepared 8/18/92 in Nanopure water (48-HRS old at 8/20/92).

SPRINGBORN LABORATORIES, INC

Study #: 1852.0602.6102-120 6103 170 | Test Material: Crotonaldehyde | Page #: 69

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: D Mgmc Chronic FHM ELS | Test Day: Day 1 Day 5 | Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Nanopure

Sampling Date: 8-31-92 Time: 1000 Sampling Source: syringe

Extraction/Dilution Date: 8 31-92 Initials: jc Authorization to Sample: AOP

Sample Identification Number	Nominal Conc. (mg/ml)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
8-92-1411	17 mg/ml	0.0500	2.00	2.00	1ml	1ml	40,000
1412	17						
1413	17						
1414	Reagent Blank						
1415	QC 1						
1416	QC 2						
1417	QC 3						
1418	D 17 mg/ml						
1419	D 17						

8/28/92

Comments: Dsbck single mdcon 8/28/92. MB

SPRINGBORN LABORATORIES, INC

Study # 1852.0692.6402.120
1852.0692.6403.130

Test Material: Crotonaldehyde

Page # 93

AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: Daphnia magna Chronic Fish Early Life Stage

Test Day: D.magna Day 14 Fish ELS Day 5

Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Nanopure Water

Sampling Date: 9/7/92 Time: 1100 Sampling Source: Syringe

Extraction/Dilution Date: 9-7-92 Initials: JC Authorization to Sample: ACP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
9-92- 310	17①	0.0500	2.00	200	〃	〃	4000
311	17①	↓	↓	↓	↓	↓	↓
312	17①						
313	Reagent Blank						
314	QC#1						
315	QC#2						
316	QC#3						
317	17②						
318	17②						

9/14/92

Comments: ① stock prepared 9/7/92 (0 hours old). ACP 9/14/92
② stock prepared 9/5/92 (48-hours old). ACP 9/14/92

SPRINGBORN LABORATORIES, INC

Study #: 1852.0692.6103.130
1852.0492.6102.120

Test Material: Crotonaldehyde

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AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 21-Day Life Cycle w/ D. magna
Fathead minnow ELS

Test Day: Day 21 D.m.
Day 12 FHMELS

Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other dilution

Sampling Date: 9/14/92 Time: 1230 Sampling Source: Syringe

Extraction/Dilution Date: 9-14-92 Initials: JC Authorization to Sample: ASP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol/Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
9-92-857	17①	0.0500	2.00	200	10	10	4000
858	17①						
859	17①						
860	Reagent Blank						
861	QC#1						
862	QC#2						
863	QC#3						
864	17②						
865	17②	↓	↓	↓	↓	↓	↓

9/14/92

Comments: ① Stock prepared 9/14/92 (0 hours old). ASP 9/14/92
② Stock prepared 9/7/92 (7 Days old). ASP 9/14/92

SPRINGBORN LABORATORIES, INC

Study #: 852.0692.6103.120
1852.0692.6102.120

Test Material: Crotonaldehyde

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AQUATIC TOXICOLOGY DEPARTMENT
SAMPLING AND PROCESSING OF CHEMISTRY SAMPLES

Test Type: 21-Day Life-Cycle w/ D.magna From Early Life-Stage

Test Day: Day 28 D.magna Day 19 Fish

Sample Type: Gravity Feed Tank Water ☐

Hard Reconstituted Water ☐ Soft Reconstituted Water ☐ Unfiltered Seawater ☐ Filtered Seawater ☐ AAP ☐ Other Nanopure

Sampling Date: 9/24/92 Time: Sampling Source: Syringe

Extraction/Dilution Date: 9-21-92 JV Initials: JV Authorization to Sample: ASP

Sample Identification Number	Nominal Conc. (mg/mL)	1° Sample Vol./Mass (mL)	Ext. Solvent Vol. (x) (mL)	1° Final Volume (mL)	2° Initial Volume (mL)	2° Final Volume (mL)	Conc. Factor (VF/VI)
9-92- 1388	17 ①	0.2500	2.00	2.00	1.00	1.00	4 & 00
1389	17 ①	↓	↓	↓	↓	↓	↓
1390	17 ①						
1391	17 ①						
1392	17 ①						
1393	17 ①						
1394	17 ①						
1395	17 ①						
1396	17 ①						
	17 ④						
	17 ④ Not Sampled						
	17 ④ ASP 9/24/92						

Comments: ① Stock #4 prepared 8/24/92 (28 Days old). ASP 9/24/92
② Stock #5 prepared 8/26/92 (26 Days old). ASP 9/24/92
③ Stock #7 prepared 8/30/92 (22 Days old). ASP 9/24/92
④ ~~Stock #6 prepared 9/14/92~~ Not sampled 9/24/92

SPRINGBORN LABORATORIES, INC

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SPRINGBORN LABORATORIES, INC.
MEAN & STANDARD DEVIATION SUMMARY
TEMPLATE: MEANS.PRG VERSION: 7/25/91

STUDY NUMBER:	1852.0692.6103.130	DATA ENTRY BY:	HJB
SPONSOR:	Eastman Kodak Co.	DATE ENTERED:	9/24/92
TEST MATERIAL:	Crotonaldehyde	SPECIES:	Daphnia magna
PARAMETER:	Hardness		

CONCENTRATION:	Control	0.094	1.5
REPLICATE:	All	All	All
MEAN =	166.4	167.2	165.6
S.D. =	6.066	1.789	5.367
N =	5	5	5
MIN =	160	164	160
MAX =	176	168	172

CONCENTRATION:	Control	0.094	1.5
REPLICATE:	All	All	All
OBSERVATION			
1	160	164	168
2	164	168	160
3	164	168	160
4	168	168	172
5	176	168	168

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SPRINGBORN LABORATORIES, INC.
MEAN & STANDARD DEVIATION SUMMARY
TEMPLATE: MEANS.PRG VERSION: 7/25/91

STUDY NUMBER:	1852.0692.6103.130	DATA ENTRY BY:	MJB
SPONSOR:	Eastman Kodak Co.	DATE ENTERED:	9/24/92
TEST MATERIAL:	Crotonaldehyde	SPECIES:	Daphnia magna
PARAMETER:	Alkalinity		

CONCENTRATION:	Control	0.094	1.5
REPLICATE:	All	All	All
MEAN =	110.8	110.8	111.6
S.D. =	1.789	1.095	2.191
N =	5	5	5
MIN =	110	110	108
MAX =	114	112	114

CONCENTRATION:	Control	0.094	1.5
REPLICATE:	All	All	All
OBSERVATION			
1	110	110	108
2	110	110	112
3	110	112	114
4	114	110	112
5	110	112	112

SPRINGBORN LABORATORIES, INC.
MEAN & STANDARD DEVIATION SUMMARY
TEMPLATE: MEANS.PRG VERSION: 7/25/91

STUDY NUMBER:	1852.0692.6103.130	DATA ENTRY BY:	NJB
SPONSOR:	Eastman Kodak Co.	DATE ENTERED:	9/28/92
TEST MATERIAL:	Crotonaldehyde	SPECIES:	Daphnia magna
PARAMETER:	Temperature		

CONCENTRATION:	Control	0.094	0.19	0.38	0.75	1.5
REPLICATE:	All	All	All	All	All	All
MEAN =	19.932	19.932	19.932	19.932	19.932	19.932
S.D. =	0.661	0.661	0.661	0.661	0.661	0.661
N =	44	44	44	44	44	44
MIN =	19	19	19	19	19	19
MAX =	21	21	21	21	21	21

CONCENTRATION:	Control	0.094	0.19	0.38	0.75	1.5
REPLICATE:	All	All	All	All	All	All
OBSERVATION						
1	21	21	21	21	21	21
2	21	21	21	21	21	21
3	21	21	21	21	21	21
4	21	21	21	21	21	21
5	21	21	21	21	21	21
6	20	20	20	20	20	20
7	19	19	19	19	19	19
8	19	19	19	19	19	19
9	19	19	19	19	19	19
10	19	19	19	19	19	19
11	19	19	19	19	19	19
12	19	19	19	19	19	19
13	20	20	20	20	20	20
14	21	21	21	21	21	21
15	21	21	21	21	21	21
16	21	21	21	21	21	21
17	20	20	20	20	20	20
18	20	20	20	20	20	20
19	20	20	20	20	20	20
20	20	20	20	20	20	20
21	20	20	20	20	20	20
22	20	20	20	20	20	20
23	20	20	20	20	20	20
24	20	20	20	20	20	20
25	20	20	20	20	20	20
26	20	20	20	20	20	20
27	19	19	19	19	19	19
28	19	19	19	19	19	19
29	19	19	19	19	19	19
30	19	19	19	19	19	19
31	20	20	20	20	20	20
32	20	20	20	20	20	20
33	20	20	20	20	20	20
34	20	20	20	20	20	20
35	20	20	20	20	20	20
36	20	20	20	20	20	20
37	20	20	20	20	20	20
38	20	20	20	20	20	20
39	20	20	20	20	20	20
40	19	19	19	19	19	19
41	20	20	20	20	20	20
42	20	20	20	20	20	20
43	20	20	20	20	20	20
44	20	20	20	20	20	20

SPRINGBORN LABORATORIES, INC.
MEAN & STANDARD DEVIATION SUMMARY
TEMPLATE: MEANS.PRG VERSION: 7/25/91

STUDY NUMBER:	1852.0692.6103.130	DATA ENTRY BY:	MJB
SPONSOR:	Eastman Kodak Co.	DATE ENTERED:	9/25/92
TEST MATERIAL:	Crotonaldehyde	SPECIES:	Daphnia magna
PARAMETER:	Dissolved Oxygen		

CONCENTRATION:	Control	0.094	0.19	0.38	0.75	1.5
REPLICATE:	All	All	All	All	All	All
MEAN =	8.514	8.486	8.436	8.311	8.195	8.02
S.D. =	0.406	0.388	0.436	0.455	0.499	0.548
N =	44	44	44	44	44	44
MIN =	7.6	7.8	7.5	7.5	7.3	7
MAX =	9.4	9.4	9.4	9.4	9.3	9.1

CONCENTRATION:	Control	0.094	0.19	0.38	0.75	1.5
REPLICATE:	All	All	All	All	All	All
OBSERVATION						
1	8.2	8.1	8.1	8.1	7.9	7.8
2	8	7.9	7.9	7.9	7.7	7.4
3	7.6	7.8	7.5	7.6	7.8	7.3
4	8.4	8.2	8.5	8	7.6	7.8
5	8.2	8.3	8.1	8.1	7.6	7.8
6	8	8.2	7.8	7.8	7.4	7.3
7	8.2	8.4	8.3	8.4	8.2	8.2
8	9.1	8.8	9	8.8	8.6	8.6
9	8.5	8.8	8.7	8.6	8.8	8.6
10	8.5	8.5	8.5	8.2	7.8	7.9
11	8.8	8.8	8.9	8.6	8.7	8.7
12	8.8	8.5	8.7	8.4	8.5	8.2
13	8.6	8.6	8.5	8.3	8.6	8.3
14	8	8.1	7.9	7.9	7.8	7.5
15	8.4	8.3	8.3	8	8.1	7.8
16	8	7.9	8.1	7.9	7.9	7.5
17	8.6	8.5	8.2	8.3	8.2	7.9
18	8.2	8.1	8.3	7.9	7.8	7.6
19	9.3	9.3	9.3	9.4	9.3	9
20	9.3	9.3	9.4	9.3	9.3	9.1
21	9.4	9.4	9.3	9.3	9.2	9.1
22	9.4	9.3	9.3	9.3	9.2	9.1
23	8.8	8.7	8.7	8.6	8.5	8.5
24	8.7	8.7	8.8	8.6	8.5	8.5
25	8.8	8.7	8.7	8.5	8.5	8.6
26	8.8	8.7	8.8	8.6	8.5	8.5
27	8.4	8.4	8.3	8.2	8.1	7.7
28	8.3	8.4	8.3	8.2	7.9	7.9
29	8.4	8.4	8.3	8.2	7.8	8
30	8.4	8.4	8.3	8.2	8.2	7.7
31	8.9	8.8	8.7	8.8	8.6	8.3
32	8.8	8.8	8.8	8.7	8.5	8.3
33	8.8	8.8	8.7	8.6	8.4	8.2
34	8.8	8.8	8.7	8.7	8.5	8.2
35	8.5	8.5	8.4	8.2	8	8.2
36	8.2	7.8	7.8	7.5	7.6	7.3
37	8	8.1	8	7.8	7.3	7
38	8.4	8.1	8	8.1	8.1	8
39	8.5	8.5	8.5	8.2	8	8.2
40	8.4	8.3	8.3	8.3	8.2	7.8
41	8.4	8.3	8.2	7.9	7.9	7.4
42	8.4	8.4	8.1	7.9	8	7.3
43	8	8.3	8.1	7.9	7.6	7.4
44	8.4	8.4	8.1	7.9	7.9	7.4

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D1
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 1 DATE PRINTED: 9/1/92 AEP 9/1/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.094	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.19	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.38	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.75	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
1.5	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
	A		
	B		
	C		
	D		
	AVERAGE		
	STD. DEV.		

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	
STD. DEV.	0	

(To Pool Data, turn Model off & type Alt-F10 Pool)

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SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D2
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP ACP 9/1/92
TEST DAY: 2 DATE PRINTED: 9/1/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.094	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.19	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.38	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.75	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
1.5	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
	A		
	B		
	C		
	D		
	AVERAGE		
	STD. DEV.		

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	
STD. DEV.	0	

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D4
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 4 DATE PRINTED: 9/1/92

CONCENTRATION MG/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.094	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.19	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.38	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.75	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
1.5	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
	A		
	B		
	C		
	D		
	AVERAGE		
	STD. DEV.		

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	
STD. DEV.	0	

(To Pool Data, turn Nodel off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.07
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 7 DATE PRINTED: 9/1/92 AEP 9/1/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.094	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.19	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.38	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
0.75	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
1.5	A	100	
	B	100	
	C	100	
	D	100	
	AVERAGE	100	
	STD. DEV.	0	
	A		
	B		
	C		
	D		
	AVERAGE		
	STD. DEV.		

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	
STD. DEV.	0	

(To Pool Data, turn Model off & type Alt-F10 Pool)

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SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D9
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP AEP 9/2/92
TEST DAY: 9 DATE PRINTED: 9/2/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	7
	B	100	10
	C	100	12
	D	100	10
AVERAGE		100	10
STD. DEV.		0	2
0.094	A	100	21
	B	100	8
	C	100	11
	D	100	11
AVERAGE		100	13
STD. DEV.		0	6
0.19	A	100	7
	B	100	12
	C	100	13
	D	100	8
AVERAGE		100	10
STD. DEV.		0	3
0.38	A	100	9
	B	100	12
	C	100	11
	D	100	10
AVERAGE		100	11
STD. DEV.		0	1
0.75	A	100	11
	B	100	12
	C	100	9
	D	100	7
AVERAGE		100	10
STD. DEV.		0	2
1.5	A	100	12
	B	100	10
	C	100	10
	D	100	9
AVERAGE		100	10
STD. DEV.		0	1
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	11
STD. DEV.	0	3

(To Pool Data, turn Model off & type Alt-F10 Pool)

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SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D11
SPONSOR: Eastman Kodak DATA ENTRY BY: MJB
TEST DAY: 11 DATE PRINTED: 9/4/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	50
	B	100	42
	C	100	57
	D	100	47
AVERAGE		100	49
STD. DEV.		0	6
0.094	A	100	51
	B	100	52
	C	100	54
	D	100	51
AVERAGE		100	52
STD. DEV.		0	1
0.19	A	100	44
	B	100	54
	C	100	47
	D	100	46
AVERAGE		100	48
STD. DEV.		0	4
0.38	A	100	42
	B	100	50
	C	100	42
	D	100	45
AVERAGE		100	45
STD. DEV.		0	4
0.75	A	100	58
	B	100	55
	C	100	51
	D	100	38
AVERAGE		100	51
STD. DEV.		0	9
1.5	A	100	54
	B	100	47
	C	100	43
	D	100	46
AVERAGE		100	48
STD. DEV.		0	5
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	100	49
STD. DEV.	0	5

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D14
SPONSOR: Eastman Kodak DATA ENTRY BY: MJB
TEST DAY: 14 DATE PRINTED: 9/9/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	106
	B	100	92
	C	100	107
	D	100	94
AVERAGE		100	100
STD. DEV.		0	8
0.094	A	100	97
	B	100	110
	C	100	97
	D	100	97
AVERAGE		100	100
STD. DEV.		0	7
0.19	A	100	96
	B	100	111
	C	90	88
	D	70	77
AVERAGE		90	93
STD. DEV.		14	14
0.38	A	100	91
	B	100	98
	C	100	90
	D	100	95
AVERAGE		100	94
STD. DEV.		0	4
0.75	A	100	115
	B	100	108
	C	100	99
	D	100	74
AVERAGE		100	99
STD. DEV.		0	18
1.5	A	100	99
	B	100	95
	C	100	83
	D	100	93
AVERAGE		100	93
STD. DEV.		0	7
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	98	96
STD. DEV.	6	10

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.016
SPONSOR: Eastman Kodak DATA ENTRY BY: MJB
TEST DAY: 16 DATE PRINTED: 9/9/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	106
	B	100	92
	C	100	107
	D	100	94
AVERAGE		100	100
STD. DEV.		0	8
0.094	A	100	97
	B	100	110
	C	100	97
	D	100	97
AVERAGE		100	100
STD. DEV.		0	7
0.19	A	100	96
	B	100	111
	C	90	88
	D	70	77
AVERAGE		90	93
STD. DEV.		14	14
0.38	A	100	91
	B	100	98
	C	100	93
	D	100	95
AVERAGE		100	94
STD. DEV.		0	3
0.75	A	100	115
	B	100	114
	C	100	104
	D	100	83
AVERAGE		100	104
STD. DEV.		0	15
1.5	A	100	99
	B	100	95
	C	100	83
	D	100	97
AVERAGE		100	94
STD. DEV.		0	7
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	98	97
STD. DEV.	6	10

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D18
SPONSOR: Eastman Kodak DATA ENTRY BY: NJB
TEST DAY: 18 DATE PRINTED: 1/1/00 AER 9/4/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	171
	B	100	150
	C	100	167
	D	100	148
AVERAGE		100	159
STD. DEV.		0	12
0.094	A	100	149
	B	100	165
	C	100	152
	D	100	153
AVERAGE		100	155
STD. DEV.		0	7
0.19	A	100	145
	B	100	144
	C	90	140
	D	70	105
AVERAGE		90	134
STD. DEV.		14	19
0.38	A	100	142
	B	100	164
	C	100	145
	D	100	148
AVERAGE		100	150
STD. DEV.		0	10
0.75	A	100	171
	B	100	164
	C	100	145
	D	100	125
AVERAGE		100	151
STD. DEV.		0	21
1.5	A	100	144
	B	100	146
	C	100	128
	D	100	139
AVERAGE		100	139
STD. DEV.		0	8
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	98	148
STD. DEV.	6	15

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.021
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 21 DATE PRINTED: 9/14/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	241
	B	90	212
	C	100	241
	D	100	217
AVERAGE		98	228
STD. DEV.		5	15
0.094	A	100	207
	B	100	231
	C	100	209
	D	100	214
AVERAGE		100	215
STD. DEV.		0	11
0.19	A	100	190
	B	100	188
	C	90	199
	D	70	144
AVERAGE		90	180
STD. DEV.		14	25
0.38	A	100	202
	B	100	224
	C	100	201
	D	100	205
AVERAGE		100	208
STD. DEV.		0	11
0.75	A	100	230
	B	100	222
	C	100	199
	D	100	179
AVERAGE		100	208
STD. DEV.		0	23
1.5	A	100	196
	B	100	211
	C	100	186
	D	100	196
AVERAGE		100	197
STD. DEV.		0	10
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	98	206
STD. DEV.	7	21

(To Pool Data, turn Model off & type Alt-F10 Pool)

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SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D23
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 23 DATE PRINTED: 9/16/92 AEP 9/16/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	268
	B	90	212
	C	100	259
	D	100	218
AVERAGE		98	239
STD. DEV.		5	28
0.094	A	100	220
	B	100	244
	C	100	229
	D	100	243
AVERAGE		100	234
STD. DEV.		0	12
0.19	A	100	206
	B	90	206
	C	90	200
	D	70	174
AVERAGE		88	197
STD. DEV.		13	15
0.38	A	100	234
	B	100	245
	C	100	211
	D	100	220
AVERAGE		100	228
STD. DEV.		0	15
0.75	A	100	259
	B	90	243
	C	100	224
	D	100	200
AVERAGE		98	232
STD. DEV.		5	25
1.5	A	100	204
	B	100	235
	C	100	193
	D	100	226
AVERAGE		100	215
STD. DEV.		0	19
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	97	224
STD. DEV.	7	23

(To Pool Data, turn Model off & type Alt-F10 Pool)

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SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_08S.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIO08S.DZS
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP AEP 9/18/92
TEST DAY: 25 DATE PRINTED: 9/18/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	307
	B	90	280
	C	100	308
	D	100	280
AVERAGE		98	294
STD. DEV.		5	16
0.094	A	100	263
	B	100	296
	C	100	271
	D	100	273
AVERAGE		100	276
STD. DEV.		0	14
0.19	A	100	242
	B	90	242
	C	80	258
	D	70	193
AVERAGE		85	234
STD. DEV.		13	28
0.38	A	100	258
	B	100	286
	C	100	251
	D	100	262
AVERAGE		100	264
STD. DEV.		0	15
0.75	A	100	289
	B	90	282
	C	100	272
	D	100	241
AVERAGE		98	271
STD. DEV.		5	21
1.5	A	100	260
	B	100	269
	C	100	249
	D	100	258
AVERAGE		100	259
STD. DEV.		0	8
	A		
	B		
	C		
	D		
AVERAGE			
STD. DEV.			

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	97	266
STD. DEV.	8	25

(To Pool Data, turn Model off & type Alt-F10 Pool)

SPRINGBORN LABORATORIES, INC.
DAPHNIA MAGNA SURVIVAL & REPRODUCTION CHRONIC TEST SUMMARY
TEMPLATE: BIO_OBS.SUM VERSION: 5/31/91

STUDY NO.: 1852.0692.6103.130 TEST SPECIES: Daphnia magna
TEST MATERIAL: Crotonaldehyde FILE NAME: BIOOBS.D28
SPONSOR: Eastman Kodak DATA ENTRY BY: AEP
TEST DAY: 28 DATE PRINTED: 9/21/92

CONCENTRATION mg/L	REP.	% SURVIVAL	OFFSPRING PER FEMALE
Control	A	100	349
	B	90	341
	C	100	359
	D	100	330
	AVERAGE	98	345
	STD. DEV.	5	12
0.094	A	100	308
	B	100	340
	C	100	317
	D	100	327
	AVERAGE	100	323
	STD. DEV.	0	14
0.19	A	100	295
	B	90	304
	C	80	313
	D	70	255
	AVERAGE	85	292
	STD. DEV.	13	26
0.38	A	100	307
	B	100	339
	C	100	305
	D	100	308
	AVERAGE	100	315
	STD. DEV.	0	16
0.75	A	100	340
	B	90	341
	C	90	333
	D	100	300
	AVERAGE	95	329
	STD. DEV.	6	19
1.5	A	100	319
	B	100	329
	C	100	304
	D	100	314
	AVERAGE	100	317
	STD. DEV.	0	10
	A		
	B		
	C		
	D		
	AVERAGE		
	STD. DEV.		

TOTALS FOR TREATMENT LEVELS:

	% Survival	Offspring Per Female
AVERAGE	96	320
STD. DEV.	8	22

(To Pool Data, turn Model off & type Alt-F10 Pool)

Zeigler Brothers, Inc. Salmon Starter*		
Date Submitted:12/13/90 Date Reported: 1/10/91		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0 01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0 01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg.kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	0.04 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCB's	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.2 mg/kg	0.2
Endosulfan I	<0.01 mg/kg	0.01
Endosulfan II	<0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Lot #031891 A1 Selco Food Sample*		
Date Submitted:5/22/91 Date Reported: 6/7/91		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I;II;III;	attached	
Arsenic	< 0.1 ppm	0.1
Cadmium	< 0.2 ppm	0.2
Lead	< 0.2 ppm	0.2
Mercury	< 0.02 ppm	0.02
* Analyzed by Lancaster Laboratories, Inc.		

Lot #031891 A1 Selco Food Sample*		
Date Submitted:5/22/91 Date Reported: 6/7/91		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0.01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg.kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	< 0.01 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCB's	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.05 mg/kg	0.05
Endosulfan I	< 0.01 mg/kg	0.01
Endosulfan II	< 0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Ankistrodesmus Suspension Grab Liquid Sample*		
Date Submitted:4/29/92 Date Reported: 5/11/92		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	< 0.1 mg/l	0.1
Cadmium	< 0.005 mg/l	0.005
Lead	< 0.05 mg/l	0.05
Mercury	0.0004 mg/l	0.0002
* Analyzed by Lancaster Laboratories, Inc.		

Ankistrodesmus Suspension Grab Liquid Sample*		
Date Submitted:4/29/92 Date Reported: 5/11/92		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
* Analyzed by Lancaster Laboratories, Inc.		

APPENDIX 4 - FOOD AND DILUTION WATER ANALYSES

ES-92-044

STUDY TITLE

CROTONALDEHYDE - THE TOXICITY TO FATHEAD MINNOW (*Pimephales promelas*) DURING AN EARLY LIFE-STAGE EXPOSURE

(In Accordance with Guideline #797.1600)

HAEL No. 92-0072 KAN 901878 CAS No. 4170-30-3

FINAL REPORT

AUTHOR

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PERFORMING LABORATORY

Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571

LABORATORY PROJECT ID

SLI Report # 92-10-4472

SLI Study #1852.0692.6102.120

STUDY SPONSOR:

Eastman Kodak Company

STUDY COMPLETION DATE

24 November 1992

QUALITY ASSURANCE STATEMENT

The raw data and report for **"Crotonaldehyde - The Toxicity to Fathead Minnow (*Pimephales promelas*) During an Early Life-Stage Exposure"** were inspected by the Springborn Laboratories, Inc., Environmental Sciences Division, Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. Dates of study inspections and dates reported to the Study Director and to Management are provided below.

It is the opinion of the QAU that this report accurately reflects the raw data collected during this study.

Inspection Date	Phase(s) Inspected	Reported to Study Director	Reported to Management
9/14/92	Stock Preparation	9/14/92	9/25/92
9/18/92	Test Material Usage Log	9/21/92	9/25/92
10/5/92	Test Termination	10/6/92	10/9/92
10/26,27/92	Raw Data	10/26,27/92	11/6/92
11/6/92	Draft Report	11/6/92	11/6/92
11/23/92	Final Report	11/23/92	11/24/92
11/24/92	Final Report	11/24/92	11/24/92

SPRINGBORN LABORATORIES, INC.

Patricia D. Royal
Manager, Regulatory Affairs
and Quality Assurance Unit

11/24/92
Date

GOOD LABORATORY PRACTICE STATEMENT

To the best of my knowledge and belief, this study was conducted according to : Good Laboratory Practice Regulations for Nonclinical Laboratory Studies as promulgated by the Environmental Protection Agency Good Laboratory Practice Standard 40 CFR, Part 792, November 29, 1983 (revised August 17, 1989); with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. In addition, analyses of the dilution water used during this study for total suspended solids concentration, chlorine residue concentration, total organic carbon concentration and chemical oxygen demand concentration were also performed by Lancaster Laboratories. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Stability, characterization and verification of the test article identity and maintenance of records on the test article are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test article will be sent to the Study Sponsor. Archival of a sample of the test article is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

Mark W Machado 11/24/92

Mark W. Machado Date
Study Director

EASTMAN KODAK COMPANY

Joseph W. Gorsuch 11/25/92

Joseph W. Gorsuch Date
Sponsor's Representative

TABLE OF CONTENTS

STUDY TITLE

Crotonaldehyde - The Toxicity to Fathead Minnow (*Pimephales promelas*) During an Early Life-Stage Exposure

ABSTRACT

The purpose of this study was to estimate the chronic toxicity of crotonaldehyde to fathead minnow (*Pimephales promelas*) under flow-through conditions. Fathead minnow embryos/larvae were continuously exposed for 33 days (28 days post-hatch) to nominal concentrations of 1.7, 0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L and a dilution water control. Observations were made on the percentage of healthy, fertile embryos following 40- to 48-hours of test initiation. Observations of survival of organisms at hatch and on survival and growth (total length and wet weight) of larvae after 28 days of post-hatch exposure were made.

Analyses of test article stock solutions were performed on days 0, 5, 12, 19, 26 and 33. Results of these analyses confirmed that concentrations of crotonaldehyde in the stock solutions were close to nominal. Reported early life-stage results are based on nominal concentrations. Exposure solutions were observed to be clear and colorless throughout the exposure. Analysis of the water quality parameters (e.g., temperature, dissolved oxygen concentration, total hardness and alkalinity, etc.) throughout the exposure period established that these water quality parameters remained within acceptable limits for the survival and growth of fathead minnow.

Between 40 - 48 hours after the initiation of the definitive early life-stage test, the percent of healthy, fertile embryos was determined for each test concentration and the control. Only 5% of the organisms exposed to the 1.7 mg A.I./L test concentration were determined to be healthy, fertile embryos which was significantly different ($p \leq 0.05$) as compared to the control organisms (95%). Embryos identified to be healthy and fertile in the

remaining test concentrations (0.87 - 0.054 mg A.I./L) ranged from 83 - 93% and was similar to that of the control group.

At the completion of the hatching period (day 5), no survivors were observed among organisms exposed to the 1.7 mg A.I./L test concentration, while 73% of the organisms exposed to the 0.87 mg A.I./L test concentration survived. Statistical analysis determined that survival in the 0.87 mg A.I./L treatment level was significantly different ($p \leq 0.05$) compared to the survival of the control organisms (90%). Survival among organisms exposed to the remaining test concentrations (0.43 - 0.054 mg A.I./L) ranged from 80 - 90% and was not statistically different from the survival of the control organisms (90%). No abnormalities were observed among any of the live larvae in this test, therefore, statistical analyses to determine the percentage of embryos that produced live, normal larvae were not conducted.

Following 28-days post-hatch exposure, larval survival among organisms exposed to the 0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L crotonaldehyde was determined to be 66, 85, 69, 73 and 83%, respectively. Survival in these test concentrations was not statistically different from the survival of the control organisms (82%). No abnormalities were observed among any of the surviving larvae in this test. Therefore, statistical analyses to determine the percentage of embryos that produced live, normal larvae at the end of the test were not conducted.

Mean total lengths for organisms exposed to the 0.87, 0.43 and 0.22 mg A.I./L test concentrations were 25, 27 and 28 mm, respectively, and were statistically different ($p \leq 0.05$) when compared to the mean total length of the control larvae (29 mm). Total lengths at the remaining two concentrations tested (0.11 and 0.054 mg A.I./L) averaged 29 mm each and were statistically equivalent to the mean total lengths of the control organisms.

Mean wet weight for organisms exposed to the 0.87 mg A.I./L test concentration was 0.17 g. Statistical analysis determined a significant difference was present when this value was compared to the mean wet weights of the control organisms (0.25 g). Mean wet weights for organisms exposed to the four lowest treatment levels (0.43 - 0.054 mg A.I./L) ranged from

0.23 - 0.26 g and were not statistically different from the weight of organisms exposed to the control solutions.

Based on these results, mean larval length was determined to be the most sensitive indicator of the toxicity of crotonaldehyde to the fathead minnow. The Lowest-Observed-Effect Concentration (LOEC) was 0.22 mg A.I./L. Although the difference between the length of larvae exposed to 0.22 mg A.I./L was determined to be statistically different from the length of the control organisms (e.g., 28 mm vs. 29 mm) the biological significance of this difference is uncertain. Utilization of this difference (1 mm) to establish the Lowest-Observed-Effect Concentration should be considered a conservative estimate of the toxicity of crotonaldehyde to early life-stages of fathead minnow. The No-Observed-Effect Concentration (NOEC) was determined to be 0.11 mg A.I./L. Based on these data, the Maximum Acceptable Toxicant Concentration (MATC) for crotonaldehyde and fathead minnow was estimated to be > 0.11 mg A.I./L and < 0.22 mg A.I./L (geometric mean MATC = 0.16 mg A.I./L).

TESTING FACILITY

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SPONSOR

Eastman Kodak Company
Rochester, New York 14650
U.S.A.

DATE OF STUDY INITIATION 29 July 1992

DATES OF CHEMICAL EXPOSURE 2 September - 5 October 1992

DATE OF STUDY COMPLETION 24 November 1992

STUDY PARTICIPANTS

Mark W. Machado	Study Director
Rex Tien	Analytical Chemist
Susan P. Shepherd	Coordinator, Data Management and Reporting Unit

1.0 INTRODUCTION

1.1 Objective

The objective of this study was to determine the effects of crotonaldehyde on fathead minnow (*Pimephales promelas*) embryos and larvae during continuous aqueous exposure. Characteristics which make fathead minnow suitable for the early life-stage test are their ease in handling, their known sensitivity to a variety of toxicants, the ready availability of fertilized eggs, and the extensive existing data for this species. The definitive embryo/larval exposure was initiated within 24 hours after egg fertilization and continued through 33 days (28 days post-hatch). The effects on embryo viability following 40 to 48 hours after test initiation, on embryo survival (live larvae and live, normal larvae) at hatch, and on survival (live fish and live, normal fish) and growth (wet weight and total length) of larvae at test termination were measured and used to estimate the Maximum Acceptable Toxicant Concentration (MATC). The MATC is defined as the concentration range encompassing the highest test concentration that had no significant ($p \leq 0.05$) effect on the test organism performance and the lowest concentration that significantly affected the exposed organisms. The MATC is expressed as the geometric mean of the lowest effect and highest-observed-no effect concentrations and is estimated from the most sensitive of the criteria used (e.g., organism survival at hatch, larval survival and larval growth at test termination).

1.2 Rationale

Macek and Sleight (1977) and McKim (1977) described fish embryo/larval investigations as providing reasonably accurate short-term predictions of potential long-term chemical toxicity to fish. In the majority of the chronic toxicity studies reported by these authors, and of those performed at this laboratory, the embryos and larvae were generally the most sensitive life-stages to chemical exposure. Rarely were reproduction or survival and growth of second generation larvae reduced at exposure levels lower than those that reduced survival or growth of the first generation larvae. These authors demonstrated that for the majority of chemicals the shorter and more economical embryo/larval tests yielded estimates of safe concentrations very similar to those derived from full life-cycle chronic toxicity studies.

2.0 TEST ARTICLE

Identity: Crotonaldehyde (MSDS, Appendix 1)

Sample Reference Identification No.: Lot #7-92, CAS #4170-30-3

Received at SLI: 23 July 1992

Physical/Chemical Properties

Purity: 93.8% active ingredient (Purity Determination, Appendix 2)

Composition: The test article was received as a clear liquid

Storage Conditions: Refrigerated at 4 °C

The test-article was kept in a tightly sealed container, and any head space was purged of air using nitrogen. This was done to minimize the potential for oxidation.

Carrier Solvent: The test article stock solutions were prepared in NANOpure® water without the use of a carrier solvent.

3.0 MATERIALS AND METHODS

3.1 Protocol

The embryo-larval exposure was conducted according to the protocol entitled "Protocol for Conducting Early Life-Stage Toxicity Test with Fathead Minnow, *Pimephales promelas*, Following TSCA Guideline 797-1600", SLI Protocol #072292/TSCA 797-1600 FM-ELS/KODAK and Protocol Amendments #1 and #2 dated 20 August and 3 November 1992, respectively (Appendix 3). The procedures outlined in this protocol followed the TSCA Test Standard § 797.1600 (U.S. EPA. 1985, 1987. Toxic Substances Control Act Test Guidelines. Federal Register 50(188), September 27, 1985. Amended, May, 1987), and conformed to the consent order established between Eastman Kodak Company and U.S. EPA entitled "Testing Consent Order, Crotonaldehyde" Docket # OPTS 42108). The study was initiated on 29 July 1992, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the early life-stage

exposure was conducted from 2 September to 5 October 1992, at Springborn Laboratories, Inc. (SLI), Environmental Sciences Division, Wareham, Massachusetts.

3.2 Test Organism

Fathead minnow eggs were obtained from brood stock maintained at Springborn Laboratories, Inc. (SLI). Springborn Laboratories, Inc. has maintained a continuing reproducing culture of fathead minnows since 1973. Brood stock fathead minnow ranged from 8 - 13 months old at the time of egg collection for this study and were judged to be in good health. On the day prior to test initiation, spawning tiles were placed in the fathead minnow brood culture unit. The eggs were collected from the tiles using the finger-rolling method and impartially distributed to the egg incubation cups in the following manner: fourteen unlabeled, unassigned petri dishes were set in a shallow waterbath maintained at 25 °C. A small amount of water from the control aquaria was placed in each dish. The collected eggs were then counted into each dish sequentially, five at a time, until each dish contained forty eggs. Subsequently, a second count was conducted of the eggs to ensure accuracy. Fourteen labeled incubation cups were placed in control water at 25 °C. Each group of 40 eggs in the petri dishes was impartially transferred to one of the fourteen incubation cups. At test initiation, the embryos were ≤24 hours old.

Following the completion of hatching (day 5), the larvae were fed live brine shrimp nauplii (*Artemia salina*, ≤48 hours post-hydration) three times daily on weekdays and two times daily on weekends. During the first seven days of feeding, food was added at minimum intervals of four hours (i.e., 8:30 am, 12:30 pm and 4:30 pm). Representative samples of the brine shrimp nauplii were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix 4). The food was considered to be of acceptable quality since the total concentration of pesticides measured was less than 0.3 mg/kg (ASTM, 1985).

3.3 Test Dilution Water

The dilution and control water used during this study was well water which was pumped into an epoxy-coated concrete reservoir where it was supplemented on demand with Town of Wareham untreated well water and aerated. During the study, weekly characteriza-

tion of the well water established total hardness and total alkalinity ranges as $CaCO_3$ of 24 - 29 mg/L and 20 - 25 mg/L, respectively, a pH range of 6.9 - 7.1, and a specific conductivity range of 100 - 130 μmhos/cm. Representative samples from the dilution water source were also analyzed for the presence of pesticides, PCBs, and toxic metals (Appendix 4). None of these compounds were detected in any of the water samples analyzed at concentrations that are considered toxic, in agreement with U.S. EPA and ASTM guidelines. Several species of daphnids (a representative freshwater organism generally recognized to be sensitive to chemical changes) are maintained in water from the same source as the dilution water used in the study and have successfully survived and reproduced over several generations. This, in combination with the previously mentioned analyses, confirms the acceptability of the dilution water for toxicity studies. In addition, representative samples of the dilution water source used during this study were also analyzed monthly for total organic carbon (TOC) concentration, chemical oxygen demand (COD) concentration, total suspended solids (TSS) concentration, unionized ammonia concentration and residual chlorine concentration. Based on these analyses, the dilution water source was determined to have a TOC concentration within the range of 0.5 - 0.9 mg/L, a COD concentration of ≤ 7 mg/L, a TSS concentration of ≤ 4 mg/L, an unionized ammonia concentration of ≤ 0.1 mg/L and a residual chlorine concentration of < 0.05 mg/L.

3.4 Stock Solution

A study was conducted to determine the stability of crotonaldehyde in three different freshwater matrices: soft water (hardness approximately 26 mg/L as $CaCO_3$), hard reconstituted water (hardness approximately 170 mg/L as $CaCO_3$) and NANOpure® water (ASTM Type II). Test solutions were prepared by fortifying each water type with the appropriate amount of crotonaldehyde to achieve a nominal concentration of 17 mg A.I./mL. An aliquot of each solution was removed for analysis immediately upon fortification (hour 0). Subsequent samples were removed after 24 and 48 hours. An additional sample was removed from the test solution prepared in NANOpure® water after 96 hours. All samples were analyzed according to the methodology described in Appendix 5.

Results of analyses conducted at 0 and after 24 hours indicated that crotonaldehyde was stable in NANOpure® water but was not stable in either soft or hard reconstituted water (Table 1). Average measured concentrations of crotonaldehyde solutions prepared in hard reconstituted water averaged only 14% of the nominal fortified concentration at 0 hour and were variable at 24 hour with a mean of 13% of nominal (range; 8 to 18%). Measured concentrations in soft water averaged 75% of the nominal fortified concentration at 0 hour, but decreased to a maximum of 10% of nominal within 24 hours. Analysis of test solutions prepared in NANOpure® water at 0 hour and 24 hours resulted in measured concentrations which averaged 87% and 84% of the nominal fortified concentration, respectively.

Analytical difficulties were experienced during the preparation and the analysis of the experimental samples removed at 48 hours. Based on the data obtained during the first 24 hours, no further evaluation of the soft or hard reconstituted water matrices was conducted; however, additional samples were removed from the NANOpure® water test solution after 96 hours to confirm the stability of crotonaldehyde in this matrix. The results of these additional analyses (e.g., measured concentrations averaging 106% of nominal) at 96 hours indicate that crotonaldehyde was stable in NANOpure® water for at least 96 hours. Based on these data, all stock solutions of crotonaldehyde used to prepare exposure solutions for the effects tests were prepared in NANOpure® water.

The consent order specified that stock solution analysis was to be conducted every 7 days throughout the exposure period. A summary of the day of stock preparation, the day of stock analysis and the age of representative stock solutions is presented below.

Day of Preparation	Day Sampled and Analyzed	Age of Stock (days) at Time of Analysis
-1	0	1
3	NA	NA
5	5	0
12	12	0
19	19	0
26	26	0
26	33	7

NA = Not Applicable

Table 3 presents the results of the analysis of the stock solutions for crotonaldehyde concentration performed on days 0, 5, 12, 19, 26 and 33.

Analysis of stock solutions during the in-life portion of the fathead minnow early life-stage study and the life-cycle study with *Daphnia magna* (SLI Report #92-10-4473) further indicated that the crotonaldehyde stock solutions (prepared in NANOpure® water) were generally stable for 7 days. Although some variability is apparent in the individual measured values, the average measured concentrations resulting from analyses conducted on 7-day old stock solutions did not differ significantly relative to the nominal concentrations. Measured stock solution concentrations and diluter calibrations established that crotonaldehyde was being delivered to the exposure system at concentrations consistent with the nominal treatment levels.

Stock solutions of 17 mg A.I./mL were prepared on exposure days -1 and 3 by diluting approximately 3.6520 g (3.4256 g active ingredient) of test article with NANOpure® water (which had been previously purged with nitrogen for 3 - 4 minutes prior to use) to a volume of 200 mL (Chemical Distribution Record, Appendix 6). Stock solutions of 17 mg A.I./mL

diluter stock were prepared on test days 5, 12, 19 and 26 by dissolving approximately 9.1310 g (8.5649 g active ingredient) of test article with NANOpure® water (also purged with nitrogen for 3 - 4 minutes) to a volume of 500 mL. Diluter stocks were observed to be clear and colorless.

3.5 Test Conditions

The test was conducted using an exposure system consisting of an intermittent-flow proportional diluter (Mount and Brungs, 1967), a temperature-controlled water bath and a set of 14 exposure aquaria. The test system was designed to provide six concentrations of the test article and a dilution water control. A Sage syringe pump, in conjunction with a 50-mL Glenco® gas-tight syringe, was calibrated to deliver 0.23 mL of the stock solution (17 mg A.I./mL) during each diluter cycle into the diluter's chemical mixing chamber which contained 2.25 L of dilution water. The solution contained in the mixing chamber constituted the highest test concentration (1.7 mg A.I./L, nominal) and was subsequently diluted (50%) to provide the range of nominal exposure concentrations (0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L). Calibration of the diluter system was confirmed prior to test initiation, weekly during the study and following test termination. All treatment levels and the control were maintained in duplicate. Test aquaria were labeled to identify the nominal test article concentration and designated replicate. Beginning on test day 21, the column saturator, which was installed on the diluter system prior to test initiation, was turned on in order to aerate the inflowing dilution water. The individual exposure solutions were not aerated at any time during the exposure. Illumination was provided by Durotest Vitalite® fluorescent lights centrally located above the test aquaria. A 16-hour light and 8-hour dark photoperiod was provided daily with a light intensity range of 50 - 100 footcandles at the surface of the exposure solutions. Sudden transitions from light to dark and vice versa were avoided.

The diluter system and exposure aquaria were fabricated of glass and silicone sealant. Each test aquarium measured 39 x 20 x 25 cm with a 14.5 cm high side drain that maintained a constant exposure solution volume of 11 L. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms. A rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the incubation cups in the test solutions. The

diluter delivered the control and test solutions to the exposure aquaria at a rate sufficient to provide approximately 6.7 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 8.0 hours (Sprague, 1969). The aquaria were impartially positioned in a waterbath containing circulating water designed to maintain the test solution temperatures at 25 ± 2 °C. During the exposure period, all aquaria were brushed and siphoned to remove detritus and uneaten food at least once weekly.

4.0 TEST PROCEDURES

4.1 Test Initiation

The exposure of fathead minnow embryos and larvae to crotonaldehyde was initiated when the egg incubation cups, each containing forty embryos, were distributed to each of the fourteen test aquaria. The diluter system was allowed to equilibrate with crotonaldehyde for eleven days prior to addition of the test organisms. A visual check of proper diluter function was performed at least twice daily throughout the study.

4.2 Test Monitoring

4.2.1 Embryo-Larval Exposure. Dead embryos were counted and removed daily until hatching was complete. Hatching was deemed complete (exposure day 5) when no more than 5 unhatched viable embryos remained in any control or treatment-level egg incubation cup. Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was completed compared to the number of embryos per cup on test day 0.

The 28-day post-hatch larval exposure was initiated when all surviving larvae in each incubation cup were removed and placed into their respective exposure aquaria. Dead larvae were removed when observed, and behavior and appearance of larvae were observed and recorded daily. Larval survival was estimated at least twice weekly. At 28-days post-hatch exposure (test termination), the percentage larval survival was determined. The surviving larvae were anesthetized with MS-222 (tricaine methane-sulfonate) and measured for mean total length, and mean wet weight. The larvae were measured and weighed individually to calculate the means and standard deviations of total length and wet weight.

4.2.2 Water Quality Measurements. Dissolved oxygen concentration, pH and temperature were measured in each aquarium daily. The temperature was continuously monitored in one replicate (A) of the dilution water control. Dissolved oxygen concentration was measured using a YSI Model #57 dissolved oxygen meter with a combination (temperature/dissolved oxygen) electrode polarographic probe. A Jenco Model 601A or a Hanna Model HI pH meter and combination electrode were used for pH measurements. Temperature (daily measurement) was measured with a Brooklyn alcohol thermometer. Continuous monitoring of the control solution temperature was performed using a Brooklyn Thermometer Company Min-Max thermometer in addition to an Omega Data Acquisition System (ODAS). Total hardness, total alkalinity and total acidity as $CaCO_3$ (APHA *et al.*, 1985), specific conductance, total organic carbon (TOC) concentration and particulate matter (e.g., total suspended solids, TSS) were measured on day 0 and weekly thereafter in alternating replicates of the high and low test concentrations and the dilution water control solution. Specific conductance was measured using a YSI Model #33 conductivity meter. TOC measurements were performed with a Dohrman DC-80, and TSS measurements were performed using an 11 cm diameter Whatman 934-AH filter, which was weighed on a SP-182 analytical balance. Unionized ammonia was measured in alternating replicates of the control solution twice weekly with an Orion Model SA-720 meter and a gas-permeable probe (Model 9512). Light intensity was measured with a General Electric Model 214 light meter.

4.3 Analytical Measurements

Triplicate samples of stock solutions were analyzed on days 0, 5, 12, 19, 26 and 33 for crotonaldehyde concentration. All stock solutions were analyzed within 24 hours of preparation with the exception of stock solutions analyzed on days 0 and 33. The day 0 stock solution was 1 day old at the time of analysis, while the day 33 stock solution was 7 days old at the time of analysis. In addition, duplicate samples of stock solutions prepared on days -7, 3, 5, 12 and 19 were analyzed on days 0, 5, 12, 19 and 26, respectively in order to monitor stock stability. All samples were removed from the approximate midpoint of the volumetric flask using a volumetric pipet. Samples were derivitized and extracted immediately after sampling. Three Quality Control (QC) samples were prepared at each sampling interval

and remained with the set of stock solution samples throughout the analytical process. These QC samples were prepared in NANOpure® water at a concentration of crotonaldehyde similar to the nominal stock solution concentration. Results of the analysis of QC samples were used to judge the precision and quality control maintained during the analysis of stock solution samples. All samples were analyzed utilizing a gas chromatographic (GC) procedure according to the methodology presented in Appendix 5. A method validation study conducted at SLI prior to the initiation of the chronic test established a mean recovery of crotonaldehyde of 88.5 ± 5.8% from diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$).

5.0 STATISTICAL ANALYSES

At the termination of the study, data obtained on the percentage of healthy, fertile embryos following 40 to 48 hours of test initiation, organism survival at hatch, and larval survival and larval growth (wet weight and total length at test termination) were statistically analyzed to establish significant differences between the treatment-level and control organisms. Analyses were performed using the mean organism response in each replicate aquarium rather than individual response values. All statistical conclusions were made at the 95% level of certainty except in the case of the Bartlett's Test, in which the 99% level of certainty was applied. The following procedures were used:

1) The percentage survival data were transformed (e.g. arcsine square-root percentage) for analysis.

2) The Shapiro-Wilks test for normality (Weber *et al.*, 1989) was conducted and compared the observed sample distribution with a normal distribution. The assumption that observations are normally distributed must be validated before subsequent analyses, following parametric procedures, can be performed. If the data are not normally distributed, then a non parametric procedure (e.g., Kruskal-Wallis test) is used for subsequent analyses.

3) As a check on the assumption of homogeneity of variance, implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985).

4) For each endpoint, the performance at each treatment level of crotonaldehyde was compared with the performance of the dilution water control using the Williams' Test (Williams, 1971, 1972). A description of each of this procedure is presented in Appendix 7.

5) Data obtained for organism survival at hatch and larval survival at test termination were analyzed before larval growth (length and weight). Treatment levels that cause significant survival effects at test termination are generally excluded from further statistical analysis.

A computer program (Gulley, et al, 1989) was used to perform the computations. The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as the Maximum Acceptable Toxicant Concentration (MATC). The MATC is equal to the geometric mean of the limits set by the lowest mean measured concentration that showed a statistically significant effect (Lowest-Observed-Effect Concentration, LOEC) and the highest mean measured test concentration that showed no statistically significant difference versus the control (No-Observed-Effect Concentration, NOEC). Based on these data, the MATC was estimated. Determination of these levels is based on the most sensitive of the performance criteria evaluated (e.g., organism survival at hatch, larval survival and growth).

6.0 DATA STORAGE AND RECORDS RETENTION

All raw data and the original final report produced for this study will be stored for a minimum of ten years in the archives of the Study Sponsor. A copy of the final report will be stored in the archives of Springborn Laboratories, Inc., Wareham, Massachusetts.

7.0 RESULTS

7.1 Preliminary Testing

Prior to the initiation of the definitive study, a preliminary exposure was conducted at SLI. During this preliminary test, fathead minnow larvae (< 14 days old) were exposed under flow-through conditions to nominal concentrations of 1.0, 0.50, 0.25, 0.12, 0.062 and 0.031 mg A.I./L. Throughout the exposure period, no undissolved test article (e.g., precipitate) was observed in any of the exposure solutions. Following 14 days of exposure, no mortality was observed among larvae exposed to any of the concentrations tested with the exception of the 0.12 mg A.I./L test concentration in which 10% mortality was observed. No sublethal effects (e.g., lethargy) were observed among surviving larvae exposed to any of the test concentrations. Based on these results, nominal concentrations of 1.7, 0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L were selected for the definitive exposure.

7.2 Definitive Test

7.2.1 Water Quality Determinations. A summary of the water quality parameters measured during the 33-day exposure of fathead minnow embryos and larvae is presented in Tables 2a and 2b. The concentrations of crotonaldehyde tested did not affect the pH, dissolved oxygen concentration, total hardness, total alkalinity and total acidity. Water samples removed from the high and low test solutions and the control solution throughout the exposure period contained mean total organic carbon (TOC) concentrations of 2.9, 2.8 and 3.4 mg/L, respectively, and in mean total suspended solids (TSS) concentrations of 3.4, 5.0 and 5.7 mg/L, respectively. Throughout the exposure period, concentrations of unionized ammonia measured in the control solutions ranged from 0.53 - 1.1 µg/L. Monitoring of the test solutions demonstrated that the test solution temperature ranged from 23 - 26 °C. The results of the water quality measurements made during this study established that conditions maintained throughout the 33-day exposure were satisfactory for the promotion of fathead minnow embryo hatchability, larval survival and growth.

7.2.2 Exposure Monitoring. A complete check of diluter function was made twice daily. Diluter calibration was checked at test initiation and weekly thereafter during the study. No deviations in calibration were observed throughout the study. The diluter system which

prepared and delivered the test solutions to the exposure vessels functioned properly during the 33-day study. Throughout the exposure period, the diluter stock solutions and the test solutions were observed to be clear and colorless.

Analyses of the stock solutions for crotonaldehyde were performed on days 0, 5, 12, 19, 26 and 33. The results of these analyses confirmed concentrations of crotonaldehyde in the stock solutions were close to nominal (Table 3). Review of these data in addition to data collected in support of the stability study indicated that the crotonaldehyde stock solutions (prepared in NANOpure® water) were generally stable for 7 days. Representative chromatograms from the analyses of the stock solution and a Quality Control sample are presented in Figures 1 and 2, respectively.

7.2.3 Biological Observations. Table 4 presents the percent of healthy, fertile embryos following test initiation, embryo survival at hatch and larval survival at test termination. Between 40 - 48 hours after the initiation of the definitive test, the percent of healthy, fertile embryos was determined in each test concentration and in the control (Figure 3). The percentage of healthy, fertile embryos in the highest test concentration (1.7 mg A.I./L) was 5% which was significantly different ($p \leq 0.05$) from the control organisms. Embryos identified to be healthy and fertile in the remaining test concentrations (0.87 - 0.054 mg A.I./L) ranged from 83 - 93%, which did not differ significantly from the control organisms.

At the completion of the hatching period (day 5), no survivors were observed among organisms exposed to the 1.7 mg A.I./L test concentration, while 73% of the organisms exposed to the 0.87 mg A.I./L test concentration survived (Figure 4). Statistical analysis determined that survival in the 0.87 mg A.I./L treatment level was significantly different ($p \leq 0.05$) compared to the survival of the control organisms (90%). Survival among organisms exposed to the remaining test concentrations (0.43 - 0.054 mg A.I./L) ranged from 80 - 90% and was not statistically different from the survival of the control organisms. No abnormalities were observed among any of the live larvae in this test, therefore, statistical analyses to determine the percentage of embryos that produced live, normal larvae was not conducted.

Because no organisms exposed to the 1.7 mg A.I./L test concentration survived, this treatment level was excluded from further statistical analyses. Following 28-days of post-hatch exposure, larval survival rates among organisms exposed to the 0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L test concentrations were determined to be 66, 85, 69, 73 and 83%, respectively (Figure 5). Comparison of these data to that of the control organisms (82% survival) established that no significant difference existed for larval survival at these treatment levels. No abnormalities were observed among any of the live larvae surviving in any of the concentrations, therefore, statistical analyses to determine the percentage of embryos that produced live, normal larvae at the end of the test were not performed.

Table 5 presents the growth (total length and wet weight) data for all organisms surviving at test termination. Mean total length measurements among organisms exposed to the 0.87, 0.43 and 0.22 mg A.I./L test concentrations were 25, 27 and 28 mm, respectively, and were statistically different ($p \leq 0.05$) when compared to the performance of the control larval (29 mm) (Figure 6). Mean total lengths among organisms exposed to the remaining concentrations tested (0.11 and 0.054 mg A.I./L) averaged 29 mm each and were not statistically different from the mean total length of the control organisms.

Mean wet weights of organisms exposed to the 0.87 mg A.I./L test concentration was 0.17 g, which was significantly different ($p \leq 0.05$) when compared to the mean wet weight of organisms exposed to the control solutions (0.25 g) (Figure 7). Mean wet weights among organisms exposed to the four lowest treatment levels tested (0.43 - 0.054 mg A.I./L) ranged from 0.23 - 0.26 g and were statistically similar to the mean wet weight of organisms exposed to the control solutions.

Based on these results, larval length was determined to be the most sensitive indicator of the toxicity of crotonaldehyde to fathead minnow. The Lowest-Observed-Effect Concentration (LOEC) was 0.22 mg A.I./L. The No-Observed-Effect Concentration (NOEC) was determined to be 0.11 mg A.I./L. Based on these data, the Maximum Allowable Toxicant Concentration (MATC) for crotonaldehyde and fathead minnow was estimated to be > 0.11 mg A.I./L and < 0.22 mg A.I./L (geometric mean MATC = 0.16 mg A.I./L).

Copies of raw data used to establish the maintained exposure conditions (e.g., water quality, test article concentration) and the concentration-effect response used to determine the reported LOEC, NOEC and MATC values for this study are presented in Appendix 8.

8.0 TEST VALIDITY

The following criteria for a valid test were met during the study:

A. Average survivability among control organisms was ≥ 80%, and the survival among organisms in any one control chamber was ≥ 70%.

B. The coefficient of variation of weights of surviving control fish in each replicate test vessel was less than 40%.

C. When compared to the performance of the control organisms, significant differences ($p \leq 0.05$) were observed for the following parameters: percent of healthy, fertile embryos within 40 to 48 hours after test initiation (1.7 mg A.I./L test concentration), embryo survival at hatch (1.7 and 0.87 mg A.I./L test concentrations), mean total length (0.87, 0.43 and 0.22 mg A.I./L test concentrations), and mean wet weight (0.87 mg A.I./L test concentration).

9.0 PROTOCOL DEVIATIONS

The following deviations to the protocol were noted:

1. Appendix I of the study protocol states that the loading in test chambers should not exceed 0.1 grams of fish per liter of test solution passing through the test chamber in 24 hours. During this study, the biomass loading at test termination was calculated to be 0.11 grams per liter per aquarium per day. This difference in the biomass loading is minimal and inconsequential and is therefore not considered to have an impact on the results of this study.

2. The study protocol states that min/max temperatures shall be recorded hourly in at least one test chamber. During this study, min/max temperatures were recorded hourly in replicate A of the control solution except during the period from 1415 hours

on 11 September 1992 to 1115 hours on 12 September 1992 due to an apparent temperature system-wide failure. However, continuous temperature monitoring of the control solution (replicate A) using a Brooklyn min/max thermometer established a temperature range of 23 - 26 °C during this period which confirmed that the proper temperature was being maintained.

10.0 CONCLUSION

During this study, statistical analyses were performed on the percentage of healthy, fertile embryos following 40 - 48 hours of the initiation of the test, the embryo hatching success and the survival and growth (mean total length and wet weight) of larval fish at test termination. Based on the results of these analyses, total length was determined to be the most sensitive indicator of the toxicity of crotonaldehyde to fathead minnow. The Lowest-Observed-Effect Concentration (LOEC) was 0.22 mg A.I./L. Although the difference between the length of larvae exposed to 0.22 mg A.I./L was determined to be statistically different from the length of the control organisms (i.e., 28 mm vs. 29 mm) the biological significance of this difference is uncertain. Utilization of this difference (1 mm) to establish the Lowest-Observed-Effect Concentration should be considered a conservative estimate of the toxicity of crotonaldehyde to early life-stages of fathead minnow. The No-Observed-Effect Concentration (NOEC) was determined to be 0.11 mg A.I./L. Based on these data, the MATC for crotonaldehyde and fathead minnow was estimated to be > 0.11 mg A.I./L and < 0.22 mg A.I./L (geometric mean MATC = 0.16 mg A.I./L).

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TABLES

Table 1. Stability of crotonaldehyde in water.

Matrix	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL) 0-Hour	24-Hour	48-Hour[a]	96-Hour
Hard water[b]	17	2.329	3.134	---[c]	NS
		2.384	1.356	---[c]	
Soft water[d]	17	12.510	1.709	1.650	NS
		13.070	---[c]	---[c]	
NANOpure® water	17	14.790	14.200	---[c]	19.600
		14.730	15.350	---[c]	16.540
	QC #1[e]	16.940	18.52	16.62	19.140
	QC #2	17.33	10.18[f]	16.79	21.910
	QC #3	25.16[f]	11.89[f]	25.180[f]	22.110

[a] Analytical difficulties were experienced during the preparation and the analysis of the experimental samples removed at 48 hours.
[b] Hardness equal to approximately 170 mg/L as $CaCO_3$.
[c] Below the limit of quantitation.
[d] Hardness equal to approximately 26 mg/L as $CaCO_3$.
[e] Quality Control samples prepared in NANOpure® water, nominal = 20.2 mg/L
[f] Percent recovery for this QC sample was outside of the standard range accepted by this laboratory (i.e., ± 3 standard deviations from the mean recovery established during the method validation/recovery study, Appendix 5).

NS = Not Sampled

Table 2a. **Daily water quality determinations made during the 33-day exposure (28 days post-hatch) of fathead minnow (*Pimephales promelas*) embryos and larvae to crotonaldehyde.**

Nominal Concentration (mg A.I./L)	Mean Dissolved Oxygen[a] (mg/L)	Mean Temperature[ab] (°C)	pH Range
1.7	9.0 (0.80)[c]	24 (0)[c]	6.9 - 7.5
0.87	8.3 (0.82)	24 (0)	6.8 - 7.3
0.43	8.4 (0.82)	24 (0)	6.8 - 7.3
0.22	8.5 (0.82)	24 (0)	6.8 - 7.3
0.11	8.4 (0.74)	24 (0)	6.8 - 7.3
0.054	8.4 (0.86)	24 (0)	6.8 - 7.3
Control	8.7 (0.92)	24 (0)	6.8 - 7.4

[a] N = 68
[b] Temperature measurements presented in this table represent daily measurements made in each exposure solution using a Brooklyn alcohol thermometer.
[c] Standard deviations of the measurements obtained during the 33-day study are presented in parentheses.

Table 2b. **Weekly water quality determinations made during the 33-day exposure (28 days post-hatch) of fathead minnow (*Pimephales promelas*) embryos and larvae to crotonaldehyde.**

Parameter		Nominal Concentration (mg A.I./L) Control	0.054	1.7
Total Hardness[a] (mg/L as $CaCO_3$)	Mean	30	31	31
	S.D.[b]	(2.5)	(3.0)	(3.3)
Total Alkalinity[a] (mg/L as $CaCO_3$)	Mean	22	23	24
	S.D.	(3.9)	(2.4)	(2.0)
Total Acidity[a] (mg/L as $CaCO_3$)	Mean	6.5	6.0	6.3
	S.D.	(1.4)	(1.5)	(3.3)
Specific Conductivity[a] (μmhos/cm)	Mean	130	140	140
	S.D.	(7.9)	(12)	(12)
Total Organic Carbon[a] (mg/L)	Mean	3.4	2.8	2.9
	S.D.	(3.2)	(1.9)	(1.7)
Total Suspended Solids[a] (mg/L)	Mean	5.7	5.0	3.4
	S.D.	(6.4)	(3.7)	(2.3)
Unionized[c] Ammonia (μg/L)	Mean	0.70	---[d]	---[d]
	S.D.	(0.28)	---[d]	---[d]

[a] N = 6
[b] S.D. = Standard Deviation
[c] N = 10
[d] Measurement not required for this treatment level.

Table 3. **Results of the analysis of the stock solutions for crotonaldehyde concentration during the 33-day exposure (28 days post-hatch) of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**

Nominal Concentration (mg A.I./mL)	Measured Concentration (mg A.I./mL)					
	Day 0[a]	Day 5[b]	Day 12[b]	Day 19[b]	Day 26[b]	Day 33[c]
17	15.5	22.9	20.0	15.1	19.2	16.2
	16.2	21.4	18.7	14.2	20.0	16.2
	17.6	22.5	20.2	14.8	19.8	
QC#1[d]	16.8	18.6	21.5	18.1	19.6	21.1
	(20.2)[e]	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)
QC#2	17.7	18.5	22.2[f]	16.7	19.8	20.2
	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)
QC#3	18.9	18.9	22.0[f]	18.5	19.5	20.0
	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)	(20.2)

[a] Stock solution was 1 day old at the time of sampling.
[b] Stock solution was prepared on the same day of the indicated sampling interval.
[c] Stock solution was 7 days old at the time of sampling.
[d] QC = Quality Control sample
[e] Nominal fortified concentration is presented in parentheses.
[f] Percent recovery is outside the standard acceptable range established by this laboratory (i.e. ± 3 standard deviations from the mean recovery established during the method validation, Appendix 5). Although two of the three QC samples analyzed on day 12 were outside of the standard acceptable range, results obtained for the stock solution at this sampling interval were considered to be representative since the results obtained for the stock solution on day 12 were consistent with the results obtained for the stock solution at the other sampling intervals.

Table 4. **Percentage of healthy, fertile embryos between 40- to 48-hours after test initiation, organism survival at hatch and survival of larvae at the termination of the 33-day exposure (28-days post-hatch) of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**

Nominal Concentration (mg A.I./L)		Healthy, Fertile Embryos (%)	Embryo Hatching Success (%)	Larval Survival at Termination (%)
1.7	A	10	0	NA[b]
	B	0	0	NA
	Mean	5[a]	0[a]	NA
0.87	A	85	65	79
	B	88	80	52
	Mean	86	73[a]	66
0.43	A	85	78	84
	B	88	85	85
	Mean	86	81	85
0.22	A	90	83	75
	B	75	78	63
	Mean	83	80	69
0.11	A	90	90	81
	B	78	70	66
	Mean	84	80	73
0.054	A	88	88	83
	B	98	93	83
	Mean	93	90	83
Control	A	98	90	86
	B	93	90	78
	Mean	95	90	82

[a] Significantly different ($p \leq 0.05$) as compared to the control organisms.
[b] NA = Not Applicable since 100% mortality was observed among organisms exposed to this treatment level at the termination of hatch (day 5).

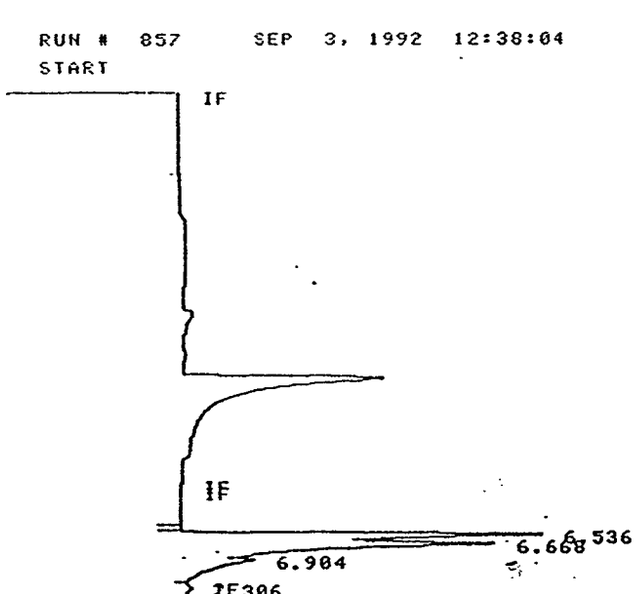
Table 5. Total length and wet weight of surviving larvae at test termination (28-days post-hatch) of the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.

Nominal Concentration (mg A.I./L)		Total Length (mm)	Wet Weight (g)
1.7	A	NA[a]	NA[a]
	B	NA	NA
	Mean	NA	NA
0.87	A	25 (1.9)[b]	0.17 (0.041)
	B	25 (2.4)	0.17 (0.051)
	Mean	25 (2.1)[c]	0.17 (0.045)[c]
0.43	A	28 (2.3)	0.24 (0.062)
	B	27 (2.3)	0.22 (0.050)
	Mean	27 (2.4)[c]	0.23 (0.057)
0.22	A	28 (2.1)	0.24 (0.054)
	B	29 (1.9)	0.25 (0.051)
	Mean	28 (2.0)[c]	0.24 (0.052)
0.11	A	28 (2.0)	0.24 (0.058)
	B	29 (1.7)	0.29 (0.058)
	Mean	29 (2.0)	0.26 (0.062)
0.054	A	29 (2.7)	0.26 (0.072)
	B	29 (3.9)	0.26 (0.087)
	Mean	29 (3.3)	0.26 (0.079)
Control	A	30 (2.8)	0.24 (0.059)
	B	29 (2.6)	0.27 (0.069)
	Mean	29 (2.7)	0.25 (0.066)

[a] NA = Not Applicable since 100% mortality was observed among organisms exposed to this treatment level at the termination of hatch (day 5).
[b] Standard deviation is presented in parentheses.
[c] Significantly different ($p \leq 0.05$) as compared to the control organisms.

FIGURES

Figure 1. Representative chromatogram showing recovery of crotonaldehyde from the stock solution prepared during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.



RUN# 857 SEP 3, 1992 12:38:04

SAMPLE NAME: 9-92-68 SAMPLE# 9
17000 MG/L

IDENTIFIER : 2728A12358

CROT

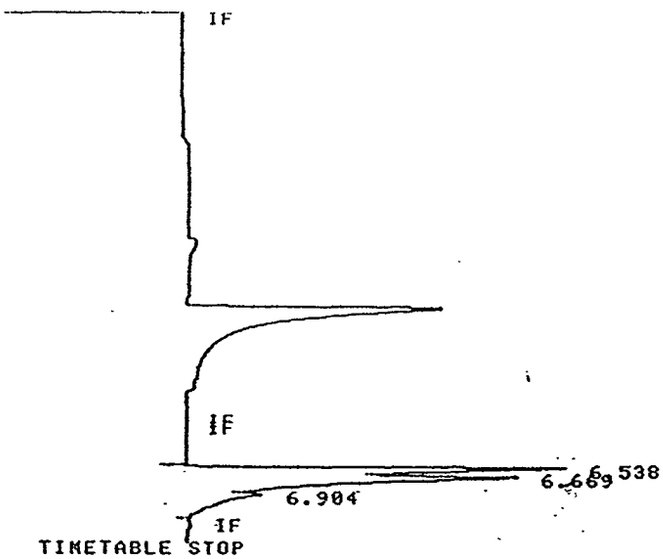
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	MG/L	NAME
6.600	++	20363808	.112	3026010	1R	45232.672	CROT
7.306	I VH	348215	.163	35509		.000	

TOTAL AREA=2.0712E+07
MUL FACTOR=4.0000E+03

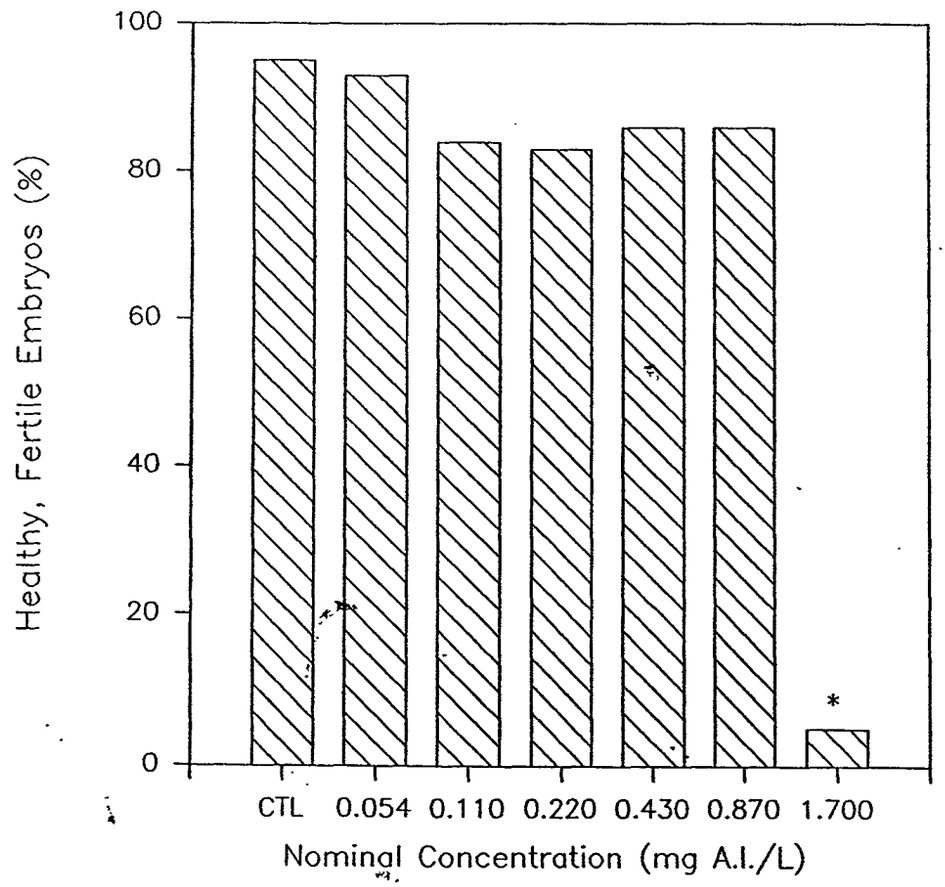
Figure 2. **Representative chromatogram showing recovery of crotonaldehyde from one of the Quality Control samples analyzed concurrently with the stock solution during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**

```
RUN #  860     SEP  3, 1992  13:08:56
START
```



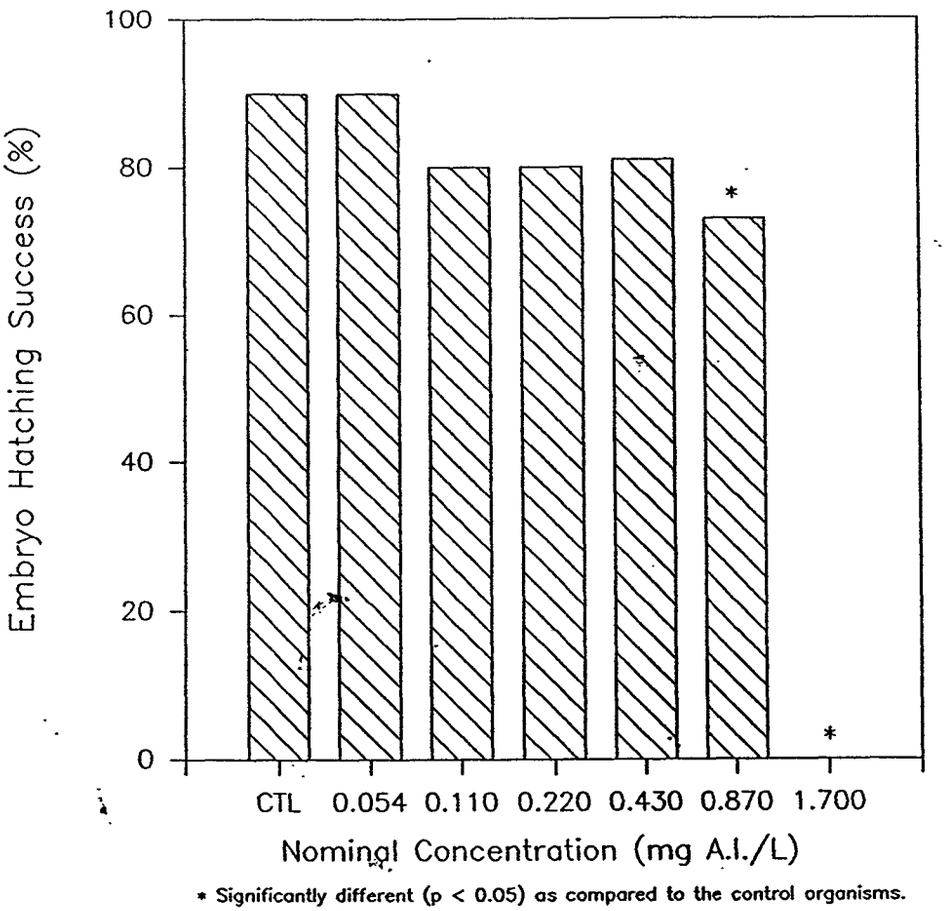

```
TIMETABLE STOP


RUN#   860          SEP  3, 1992  13:08:56

SAMPLE NAME: 9-92-71 QA1      SAMPLE#   12
20000 MG/L

IDENTIFIER : 2728A12358

CROT

ESTD%-AREA
      RT TYPE       AREA  WIDTH     HEIGHT CAL# MG/L        NAME
   6.600   ++   20971376   .111    3139163   1R   1164.532  CROT


TOTAL AREA=2.0971E+07
MUL FACTOR=4.0000E+03
SAMPLE AMT=4.0000E+03
```

Figure 3. **Percentage of healthy, fertile embryos determined following 40 - 48 hours of the initiation of the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**



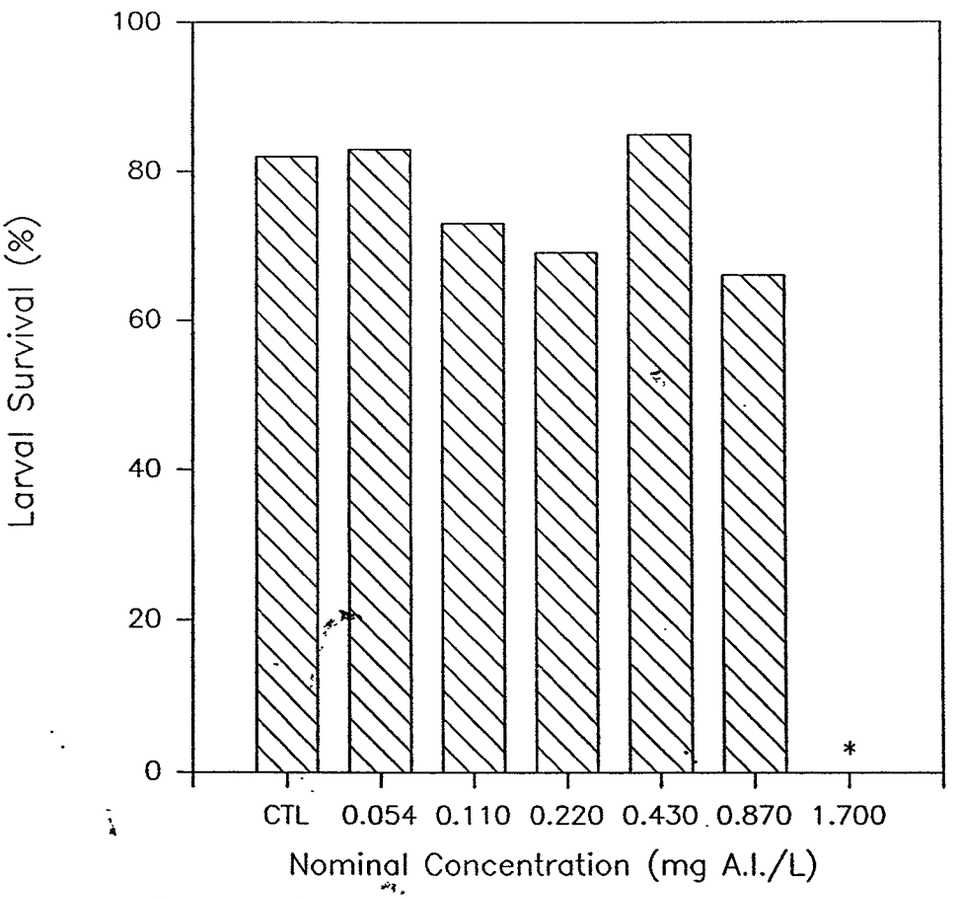
* Significantly different ($p \leq 0.05$) as compared to the control organisms.

Figure 4. **Organism survival at the completion of the hatch period (day 5) during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**



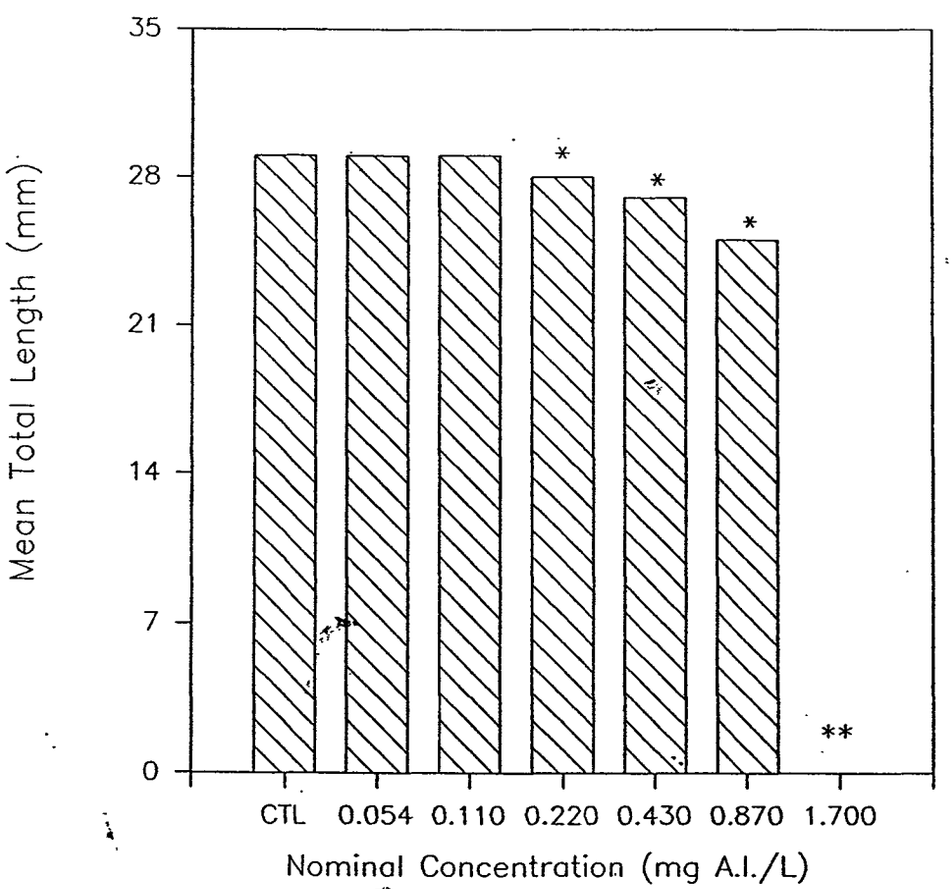
* Significantly different ($p < 0.05$) as compared to the control organisms.

Figure 5. **Larval survival during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**



* Since no organisms hatched at this treatment level, no statistical analyses were performed.

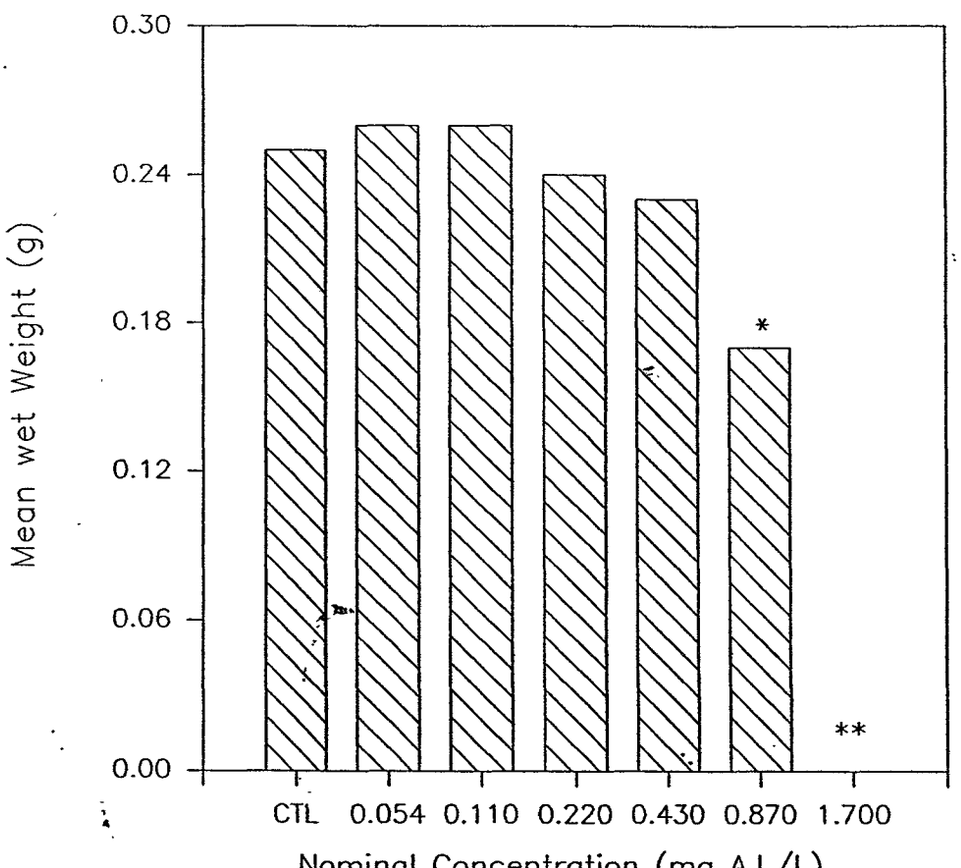
Figure 6. **Mean total length of organisms during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**



* Significantly different ($p \leq 0.05$) as compared to the control organisms.

** Since no organisms hatched at this treatment level, no statistical analyses were performed.

Figure 7. **Mean wet weight of organisms during the early life-stage exposure of fathead minnow (*Pimephales promelas*) to crotonaldehyde.**



* Significantly different ($p \leq 0.05$) as compared to the control organisms.

** Since no organisms hatched at this treatment level, no statistical analyses were performed.

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APPENDIX 1 - MATERIAL SAFETY DATA SHEET

901878

MATERIAL SAFETY DATA SHEET

EASTMAN CHEMICAL PRODUCTS, INC.
EASTMAN KODAK COMPANY
Kingsport, Tennessee 37662

For Health Hazard Information, Call: (615) 229-6094

For Other Information, Call Your Eastman Representative

Eastman Operator: (615) 229-2000 Date of Preparation 08-24-87

SECTION I. IDENTIFICATION

-- Name:

Crotonaldehyde

-- Synonyms: PM 161; 2-Butenal,
-- Formula: C_4H_6O
-- Molecular Weight: 70.09

SECTION II. PRODUCT AND COMPONENT HAZARD DATA

A. COMPONENTS:	Approx Weight %	CAS Reg No	Eastman Kodak No
Crotonaldehyde*	92	4170-30-3	901878
Water	8		

See Section VI-A for information on exposure limits.

B. PRECAUTIONARY LABEL STATEMENTS:

DANGER! FLAMMABLE
MAY BE FATAL IF INHALED OR ABSORBED THROUGH THE SKIN
CAUSES SKIN AND EYE BURNS
HARMFUL IF SWALLOWED
VAPOR EXTREMELY IRRITATING
MAY FORM EXPLOSIVE PEROXIDES
MAY POLYMERIZE

Keep away from heat, sparks, and flame.
Do not breathe vapor.
Do not get in eyes, on skin, on clothing.
Keep container tightly closed.
Use only with adequate ventilation.
Wash thoroughly after handling.
Do not allow to evaporate to near dryness.
Keep from contact with alkaline materials.

POISON-INHALATION HAZARD CALL A PHYSICIAN IMMEDIATELY

MSDS-10,597A-1 (08-87)
Replaces 07-87 Edition

FIRST AID: If inhaled, remove to fresh air. If not breathing, give artificial respiration, preferably mouth to mouth. If breathing is difficult, give oxygen. In case of contact, immediately flush eyes and skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Destroy contaminated shoes. If swallowed, DO NOT INDUCE VOMITING. If conscious, give one glass of milk or water. Never give anything by mouth to an unconscious person.

IN CASE OF FIRE: Use water spray, dry chemical, "alcohol" foam, or CO_2. Water may be ineffective in fighting the fire. Use water spray to keep fire-exposed containers cool.

IN CASE OF SPILL: Emergency personnel should wear self-contained breathing apparatus. Eliminate all ignition sources. Use water spray to disperse vapors and to flush spill area. Prevent runoff from entering drains, sewers, and streams.

Since emptied containers retain product residue, follow label warnings even after container is emptied. Do not cut, drill, grind, or weld on or near
this container.

FOR MANUFACTURING USE ONLY

SECTION III. PHYSICAL DATA (1)

-- Appearance and Odor: Clear, colorless liquid; pungent, suffocating odor; lachrymator.
-- Boiling Point: 84°C (183°F).
-- Specific Gravity (H_2O = 1): 0.871.
-- Vapor Pressure: 32 mm Hg at 20°C.
-- Percent Volatile by Volume: Approx 1.0.
-- Vapor Density (Air = 1): 2.41.
-- Evaporation Rate (ethyl ether = 1): 0.2.
-- Solubility in Water: Appreciable.

SECTION IV. FIRE AND EXPLOSION HAZARD DATA (1)

-- Flash Point: 7°C (45°F); Method Used: Tag Closed Cup.
-- Autoignition Temperature: 160°C (320°F); Method Used: ASTM E 659.
-- Cool Flame Autoignition Temperature: 121°C (250°F).
-- Flammable Limits: LEL 2.15% at 75°F.
UEL 19.5% at 165°F.
-- Extinguishing Agent: Water spray, dry chemical, CO_2, or "alcohol" foam.
-- Special Fire-Fighting Procedures: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Water may be ineffective for fire fighting. Use water spray to keep fire-exposed containers cool.
-- Unusual Fire and Explosion Hazards: Flammable liquid (see Section VIII). At elevated temperatures, such as in fire conditions, polymerization may take place. If the polymerization takes place in a container, there is a possibility of violent rupture of the container. Vapors are heavier than air and may travel along the ground or may be moved by ventilation to an ignition source and may flash back.

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SECTION V. REACTIVITY DATA (1)

-- Stability: Stable at ambient temperatures; however, may polymerize at elevated temperatures. The material readily oxidizes to an acid and may form explosive peroxides on exposure to air.
-- Stability calculated by ASTM CHETAH 4.3: Sensitive.
Heat of decomposition: -0.71 kcal/g.
Heat of combustion: -7.48 kcal/g.
-- Incompatibility: Oxidizing and alkaline materials can cause a vigorous reaction. Also see "Hazardous Polymerization" below.
-- Hazardous Decomposition Products: As with any other organic material, combustion will produce carbon dioxide and probably carbon monoxide.
-- Hazardous Polymerization: May occur. Conditions to Avoid: Violent polymerization may occur upon contact with alkaline materials such as caustic, ammonia or amines. Polymerization will also occur at elevated temperatures.

SECTION VI. TOXICITY AND HEALTH

A. EXPOSURE LIMITS

-- OSHA Permissible Exposure Limit (PEL): 2 ppm-TWA.
-- Threshold Limit Value (TLV): 2 ppm-TWA, ACGIH, 1986-87.
-- A NIOSH industrial hygiene analytical method is available. (2)

B. EXPOSURE EFFECTS

Ingestion: Harmful if swallowed.

Inhalation: May be fatal if inhaled. Vapor causes severe upper respiratory tract irritation.

Eyes: Liquid causes severe burns. Vapor extremely irritating.

Skin: May be fatal if absorbed through the skin. Causes burns.

C. FIRST AID

Ingestion: DO NOT INDUCE VOMITING. If conscious, give one glass of milk or water. Never give anything by mouth to an unconscious person. Call a physician immediately.

Inhalation: Remove to fresh air. If not breathing, give artificial respiration, preferably mouth to mouth. If breathing is difficult, give oxygen. Call a physician immediately.

Eyes: Immediately flush with plenty of water for at least 15 min. Call a physician.

Skin: Immediately flush with plenty of water for at least 15 min while removing contaminated clothing and shoes. Call a physician immediately. Wash contaminated clothing before reuse. Destroy contaminated shoes.

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Replaces 07-87 Edition

D. TOXICITY DATA

Test	Species	Result	Toxicity Classification (3)
Acute oral LD_{50}	Rat	300 mg/kg (4)	Moderately toxic
Dermal LD_{50}	Rabbit	150 to 200 mg/kg (4)	Moderately toxic
Dermal LD_{50}	Rabbit	380 mg/kg (4)	Slightly toxic
Dermal LD_{50}	Guinea pig	500 to 1000 mg/kg (4)	
Inhalation LC_{50}	Rat	600 ppm/0.5 h (5)	
Inhalation LC_{50}	Rat	380 ppm/1 h (5)	
Inhalation LC_{50}	Rat	85 ppm/4 h (5)	Highly toxic
Eye irritation	Rabbit	Severe (4)	

SECTION VII. VENTILATION AND PERSONAL PROTECTION

A. VENTILATION:

Good general ventilation (typically 10 air changes per hour) should be used. Ventilation rates should be matched to conditions. Normally, local exhaust ventilation or an enclosed handling system will be needed to control airborne levels below recommended exposure limits (see Section VI-A).

B. RESPIRATORY PROTECTION:

An appropriate full-face NIOSH-approved respirator for organic vapor must be worn if exposure is likely to exceed recommended exposure limits (see Section VI-A). If respirators are used, a program should be established to assure compliance with OSHA Standard 29 CFR 1910.134.

C. SKIN AND EYE PROTECTION:

Wear safety glasses with side shields (or goggles) and a face shield. Impermeable gloves should be worn. An impermeable apron or smock and boots should be worn to minimize skin contact. A safety shower, an eye bath, and washing facilities should be available. Wash thoroughly after handling.

SECTION VIII. SPECIAL STORAGE AND HANDLING PRECAUTIONS

Material is classified as a Flammable Liquid. Keep away from heat, sparks, and flame. Keep container closed. Use with adequate ventilation. Vapors are heavier than air and may travel along the ground or may be moved by ventilation to an ignition source and flash back. Possible peroxide former. Do not evaporate to near dryness. Keep container tightly closed. Do not contaminate. Since emptied containers retain product residue, follow label warnings even after container is emptied. Do not cut, drill, grind, or weld on or near this container.

SECTION IX. SPILL, LEAK, AND DISPOSAL PRACTICES

Steps to be Taken in Case Material is Released or Spilled: Wear appropriate protective clothing (including a self-contained breathing apparatus). Eliminate all ignition sources. Small spills may be collected with absorbent materials. For large spills, use water spray to disperse vapors and to flush area. Prevent runoff from entering drains, sewers, or streams. Clean Water Act and Superfund reportable quantity (RQ): 111 Lbs.

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Waste Disposal Method: Mix with compatible chemical which is less flammable and incinerate. Observe all federal, state, and local laws concerning health and environment.

SECTION X. ENVIRONMENTAL EFFECTS DATA

A. SUMMARY

Some laboratory data and published data are available for this product, and these data (6-8) have been used to provide the following estimate of environmental impact:

This product has a moderate to high biological oxygen demand, and it may cause oxygen depletion in aquatic systems. It has a high potential to affect aquatic organisms. This product is biodegradable and is not expected to persist in the environment. The direct, instantaneous discharge to a receiving body of water of an amount of this product which will rapidly produce by dilution a final concentration of 0.13 mg/L or less is not expected to have any adverse environmental impact. After dilution with a large amount of water, followed by secondary waste treatment, this product is not expected to have any adverse environmental impact.

B. OXYGEN DEMAND DATA

-- ThOD: 2.28 g/g (6)
-- COD: 97% of ThOD (7)
-- BOD_5: 1.54 g/g (6); 37% of ThOD (7)
-- BOD_{10}: 1.30 g/g (7)

C. ACUTE AQUATIC EFFECTS

-- 96-h LC_{50}; Bluegill sunfish: 3.5 mg/L (7,8)
-- 96-h LC_{50}; Tidewater silversides: 1.3 mg/L (7,8)

SECTION XI. TRANSPORTATION

DOT Hazard Classification: Flammable liquid (Poison - Inhalation hazard).
Flashpoint: See Section IV.
Proper DOT Shipping Name: Crotonaldehyde.
UN Number: 1143.

SECTION XII. REFERENCES

1. File data, Material Safety Program, Eastman Chemicals Division, Eastman Kodak Company, Kingsport, Tennessee.

2. NIOSH Manual of Analytical Methods, 2nd Edition, Volume 5. Issued by the National Institute for Occupational Safety and Health. Washington, U. S. Government Printing Office, 1979, Method 285.

3. AM IND HYG ASSOC Q 10, 93-96 (1949).

4. G. D. Clayton and F. E. Clayton, Editors. PATTY'S INDUSTRIAL HYGIENE AND TOXICOLOGY, 3rd Revised Edition, Volume 2A. New York, Wiley-Interscience, 1981, p. 2651.

5. AM IND HYG ASSOC J 28, 561-566 (1967).

6. Unpublished data, Health and Environment Laboratories, Eastman Kodak Co., Rochester, New York.

7. K. Verschueren. HANDBOOK OF ENVIRONMENTAL DATA ON ORGANIC CHEMICALS, 2nd Edition. Van Nostrand Reinhold Company, New York, 1983, pp. 410-411.

8. J HAZARDOUS MATER 1, 303-318 (1977).

SECTION XIII. HAZARD RATINGS

	Health	Flammability	Reactivity
HMIS* Rating:	3	3	2
NFPA** Rating:	3	3	2

NOTICE: These ratings involve data and interpretations that may vary from company to company and are intended only for rapid, general identification of the magnitude of the specific hazard. TO DEAL ADEQUATELY WITH THE SAFE HANDLING OF THIS MATERIAL, ALL THE INFORMATION CONTAINED IN THIS MSDS MUST BE CONSIDERED. The customer is responsible for determining the proper personal protective equipment needed for its particular use of this material.

*Hazardous Materials Identification System's [HMIS] Revised RAW MATERIALS RATING MANUAL, National Paint & Coatings Association, Fall 1984.

**NFPA 704 Standard System for the Identification of the Fire Hazards of Materials, National Fire Protection Association, 1985.

The information contained herein is furnished without warranty of any kind. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

TX1166S/901878/R-3, S-3, F-3, C-2

MSDS-10,597A-6 (08-87)
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APPENDIX 2 - PURITY DETERMINATION

ANALYTICAL TEST REPORT

Crotonaldehyde

Accession Number: 901878 HAEL Number: 92-0072

BY

Beth Isaacs

TESTING FACILITY

Environmental Analytical Services
Chemicals Quality Services Division
Eastman Kodak Company
1100 Ridgeway Avenue
B-320 Kodak Park
Rochester, New York 14652-3615

SPONSOR

Eastman Kodak Company
B-320 Kodak Park
Rochester, New York 14652-3615

Completion Date: 09/09/92

Accession No.: 901878 HAEL No.: 92-0072

STUDY TYPE

Environmental Studies

REQUESTED BY

Kenneth A. Robillard Ph.D.

REQUEST #: 141591

TEST SUBSTANCE

Name: Crotonaldehyde
Accession No.: 901878
HAEL No.: 92-0072
Lot No.: 7-92

DATES OF EXPERIMENT

Date received: 07/21/92
Date analyzed: 08/13/92
Date reported: 08/14/92

ANALYTICAL PERSONNEL

Beth Isaacs, Laboratory Technician

ANALYTICAL DIRECTOR

Barry W. Remington

DATA STORAGE AND RECORD RETENTION

All original raw data will be archived for at least ten years by the Chemicals Quality Sevices Division B-320 of the Eastman Kodak Co., Kodak Park, Rochester, New York 14652.

Accession No.: 901878 HAEL No.: 92-0072

METHODS:

One sample was received for a purity determination. The sample was analyzed by gas chromatography (GC) using the following instrument conditions:

Instrument:	Hewlett Packard 5890
Column:	J&W; DB Wax; 30M; wide bore; 0.25um film thickness
Carrier Gas:	Helium
Column Pressure:	7 psig
Split Flow:	120 cc/min.

Temperature Program:

Initial Temp.:	50°C
Initial Hold Time:	4 min.
Rate:	10°C/min.
Final Temp.:	250°C
Final Hold Time:	7 min.

Injection Port:	250°C
Injection Type:	split
Injection Volume:	1 uL
Detector:	Flame Ionization Detector (FID)
Detector Temp.:	250°C
Diluting Solvent:	2-Propanol

Accession No.: 901878 HAEL No.: 92-0072

RESULTS

The test chemical was diluted with 2-propanol to determine the purity. This solution was then analyzed on 08/14/92 by GC/FID. The following results are the average of three injections:

mean = 99.9% 92-0072
std. dev. = 0.0000
n = 3

ANALYST Beth Isaacs DATE 8.14.92
Beth Isaacs

REVIEWED BY [signature] DATE 9/9/92

Accession No.: 901878 HAEL No.: 92-0072

ANALYTICAL QUALITY ASSURANCE INSPECTION STATEMENT
(CFR 58.35(B)(7) 792.35(B)(7) 160.35(B)(7))

STUDY: 92-0072-Z STUDY DIRECTOR:
ANALYTICAL DIRECTOR: REMINGTON, B.
KAN : 901878
CQS JOB NUMBER: 3Z13N

STUDY TYPE: ANALYTICAL TESTING FOR ENVIRONMENTAL STUDIES

(AUDITOR, QUALITY ASSURANCE UNIT) 5/5/92 DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF HQAO, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECT DATES	REQUEST NUMBER	PHASE(S) INSPECTED	STATUS REPORT DATES
09/09/92	141591	PURITY TEST REPORT INSPECTION	09/09/92

ANALYTICAL TEST REPORT

Crotonaldehyde

KAN: 901878 HAEL Number: 92-0072

BY

Beth Isaacs

TESTING FACILITY

Environmental Analytical Services
Chemicals Quality Services Division
Eastman Kodak Company
1100 Ridgeway Avenue
B-320 Kodak Park
Rochester, New York 14652-3615

SPONSOR

Eastman Kodak Company
B-320 Kodak Park
Rochester, New York 14652-3615

Completion Date: 10/07/92

KAN: 901878 HAEL No.: 92-0072

STUDY TYPE

Environmental Studies

REQUESTED BY

Kenneth A. Robillard Ph.D.

REQUEST #: 141591

TEST SUBSTANCE

Name: Crotonaldehyde
Accession No.: 901878
HAEL No.: 92-0072
Lot No.: 7-92

DATES OF EXPERIMENT

Date received: 07/21/92
Date analyzed: 09/17/92
Date reported: 09/23/92

ANALYTICAL PERSONNEL

Beth Isaacs, Laboratory Technician

ANALYTICAL DIRECTOR

Barry W. Remington

DATA STORAGE AND RECORD RETENTION

All original raw data will be transferred to the Environmental Sciences Section of the Corporate Health and Environment Laboratories of the Eastman Kodak Co., Kodak Park, Rochester, New York 14652-3617.

KAN: 901878 HAEL No.: 92-0072

METHOD:

One sample was received for a percent moisture determination. The sample was analyzed by gas chromatography (GC) with a thermal conductivity detector (TCD), using the following instrument conditions:

Instrument:	Hewlett Packard 5890
Column:	Chrompack; plot fused silica; 25m x 0.32mm; coating poraplot Q
Carrier Gas:	Helium
Column Pressure:	12 psig
Col. + Aux. Flow:	4.0 mL/min.
Reference Flow:	15 mL/min.
Split Flow:	65 mL/min.

Temperature Program:

Initial Temp.:	80°C
Initial Hold Time:	0 min.
Rate:	10°C/min.
Final Temp.:	200°C
Final Hold Time:	5 min.

Injection Port:	200°C
Injection Type:	split
Injection Volume:	1 uL
Detector:	Thermal Conductivity Detector (TCD)
Detector Temp.:	240°C
Diluting Solvent:	2-Propanol (for standards only)

KAN: 901878 HAEL No.: 92-0072

RESULTS

The test chemical was analyzed neat on 09/17/92 by GC/TCD to determine the percent moisture. The following results are the average of two injections:

Injection	% H_2O (v/v)
1	6.2
2	6.0

mean = 6.1% (v/v)
n = 2

KAN: 901878 HAEL No.: 92-0072

Signature Page:

ANALYST Beth Isaacs DATE 9.23.92
Beth Isaacs

REVIEWED BY [signature] DATE 10-7-92

KAN: 901878 HAEL No.: 92-0072

QUALITY ASSURANCE STATEMENT

ANALYTICAL QUALITY ASSURANCE INSPECTION STATEMENT
(CFR 58.35(B)(7) 792.35(B)(7) 160.35(B)(7))

STUDY: 92-0072-Z STUDY DIRECTOR:
ANALYTICAL DIRECTOR: REMINGTON, B.
KAN : 901878
CQS JOB NUMBER: 3Z13N

STUDY TYPE: ANALYTICAL TESTING FOR ENVIRONMENTAL STUDIES

(AUDITOR, QUALITY ASSURANCE UNIT) 10/6/92 DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF HQAO, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECT DATES	REQUEST NUMBER	PHASE(S) INSPECTED	STATUS REPORT DATES
10/06/92	141591	MOISTURE DETERMINATION TEST REPORT INSPECTION	10/06/92

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APPENDIX 3 - STUDY PROTOCOL

RE

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Protocol for Conducting an Early Life Stage Toxicity Test with Fathead Minnow, *Pimephales promelas* Following TSCA Guideline 797-1600.

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: Eastman Kodak Company

Address: Environmental Sciences Section, Corporate Health and Environment Laboratories
Rochester, NY 14652-3617 **Phone:** 716/588-2140

Sponsor Protocol/Project No.:

Test Substance: Crotonaldehyde

Purity: ① 93.8 % ai **CAS# or LOT#:** CAS #4170-30-3; Lot #7-92

Additional Comments and/or Modifications:

① GC mass 11/6/92

Joseph W. Parcell, July 27, 1992
Sponsor Approval Date

TO BE COMPLETED BY SLI PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc. SLI Study Number: 1852-0692-6103-100

Study Director: MARK W. MACHADO

Test Concentrations: 2.0, 0.98, 0.49, 0.25, 0.12, 0.061 PLUS CONTROL.

Solvent Used: NEWPORT WATER CAS# or LOT#: NA

Proposed Schedule: (Start) 8/26/92 (Completion) 9/30/92

Additional Comments and/or Modifications: NONE

Mark W. Machado, 7/27/92
Study Director Date

PROTOCOL FOR CONDUCTING AN EARLY LIFE STAGE TOXICITY TEST WITH FATHEAD MINNOW, *PIMEPHALES PROMELAS* FOLLOWING TSCA GUIDELINE 797-1600

INTRODUCTION

This document describes standard toxicity test procedures used in the performance of an early life stage test with fathead minnow (*Pimephales promelas*) followed at the Environmental Toxicology & Chemistry Division of Springborn Laboratories, Inc., Wareham, Massachusetts. The procedure closely follows the TSCA Test Standard § 797.1600 (U.S. Environmental Protection Agency. 1985, 1987. *Toxic Substances Control Act Test Guidelines.* Federal Register 50(188), September 27, 1985. Amended, May, 1987), and shall conform to the consent order established between Eastman Kodak Company and U.S. EPA entitled "Testing Consent Order, Crotonaldehyde" Docket # OPTS 42108). The modified test standard associated with the current order § 797.1660 is presented in Appendix I.

Early life stage toxicity tests are conducted in order to obtain an estimate of the MATC (Maximum Acceptable Toxicant Concentration). The MATC is defined as the highest toxicant concentration not causing a statistically significant effect when compared to controls on the biological parameters measured (egg hatchability, fry survival and growth) during continuous chronic exposure. This value is presented as a range encompassing the highest "no effect" concentration (NOEC) and the lowest observed effect concentration (LOEC).

MATERIALS AND METHODS

TEST ORGANISMS

1. Species - Fathead minnow (*Pimephales promelas*) are commonly used to conduct early life stage tests. Characteristics which make fathead minnow suitable for this early life stage toxicity test are their ease of handling, their known sensitivity to a variety of toxicants, the ready availability of fertilized eggs, and the extensive existing data for this species.

2. Origin - Fertilized fathead minnow eggs are obtained from broodstock maintained at Springborn Laboratories, Inc.

PHYSICAL SYSTEM

1. Construction Materials - Materials used that come in contact with the test water are glass, stainless steel, silicone adhesive, silicone stoppers and tubing, and nylon.

2. Dilution Water - Water from a 100 meter bedrock well is pumped to a concrete reservoir where it is supplemented on demand with untreated, unchlorinated, Town of Wareham well water and aerated before flowing to the exposure system through aged PVC pipe. The pH, total hardness, alkalinity, and specific conductance of this water are measured and recorded

weekly in Springborn Laboratories' GFT Laboratory Notebook. The water is characterized as being "soft" with a pH range of 6.9 - 7.2, a total hardness of 25 - 40 mg/L and a specific conductance of 80 - 150 μmhos/cm. During any one month, weekly analysis of the dilution water should show that the water quality characteristics of hardness, alkalinity and specific conductance do not vary by more than 10% from the respective monthly average and the monthly pH range should be less than 0.4 pH units. The water is heated to 25°C in a gas-fired glass coil heater prior to flowing to the diluter. At least twice a year, analyses of representative samples of dilution water are conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the fish. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with US EPA and ASTM standard practices. A historical summary is presented in Appendix II. TOC, COD, particulate matter and unionized ammonia analyses are conducted once a month in the dilution water. The TOC concentration has ranged from 0.32 to 1.8 mg/L during the last 24 months.

3. Diluter - A proportional diluter (e.g., Mount and Brungs, 1967) or a serial diluter (e.g., Benoit et al, 1982) is employed to deliver six toxicant concentrations, and a control to duplicate aquaria. Based on the solubility of the test material, the stock solution stability and the range of test concentrations, one of the following toxicant delivery systems may be used: the gas-tight syringe injector metering device; or the metering pump/predilution chamber system.

   A flow splitting chamber is used between diluter and aquaria for each concentration to promote mixing of the toxicant bearing solution and diluent water. In each of the chambers, two separate standpipe cap siphons are employed to equally split one liter of test solution between A and B replicate aquaria. The A aquaria are randomly placed on one side of the waterbath and the B aquaria on the other side.

   The calibration of the diluter system is checked prior to test initiation, weekly during the study, and after test termination. Calibration includes determining the flow rate through each chamber as well as the proportion of stock solution to dilution water delivered to each chamber. During the test, the diluter is visually inspected at least twice daily. If there is any indication during the test that the diluter calibration has changed (e.g. diluter malfunction or unexplained differences in dissolved oxygen concentration or temperature in the aquaria), calibration of necessary diluter components is checked. A test is not started until the diluter and toxicant delivery device have been observed to be properly functioning for a minimum of 72 hours. During a test, the flow rates shall not vary by more than 10% from one replicate test chamber to another.

4. Replication - Two replicates are included with each test concentration and control. Test aquaria are positioned inside the waterbath by stratified random design, and labelled by replicate and concentration (or control).

5. Cleaning - The test chamber and the diluter are cleaned prior to the initiation of each test following standard laboratory procedures. In addition, all aquaria are brushed and siphoned to remove detritus and uneaten food as needed (weekly at a minimum) during the test.

6. Test Chambers - Each aquarium is constructed of glass and silicone adhesive and measures 39 x 20 x 25 centimeters. Water depth is maintained by a constant level overflow drain 14.5 or 19.5 cm from the bottom of each test aquarium. The total test solution volume in each aquarium is thus maintained at either 11 or 15 L. Aquaria position are identified by adhesive labels stating treatment/control and the replicate.

7. Embryo Incubation Cups - Egg incubation cups are constructed from 5 cm diameter, 8 cm high, round glass jars with the bottoms cut off and replaced with Nitex 40 mesh screen. Egg cups are oscillated in the test solution by means of a rocker arm apparatus (Mount, 1968) driven by a 2-rpm electric motor.

8. Flow Rate - Flow rates are at least 6.0 aquarium volumes per 24 hours. Flow rates are selected so as to ensure that the fish biomass to solution ratio ("loading") does not exceed 0.1 grams per liter per 24 hours.

9. Temperature - Water temperature is maintained at 25 ± 2°C by resting the aquaria in a temperature controlled waterbath containing circulating water. The temperature range is monitored continuously in one test solution by using a minimum-maximum thermometer and recorded hourly.

10. Dissolved Oxygen - Total dissolved oxygen (DO) concentrations are not allowed to remain below 75% of saturation for more than 24 hours, and flow rates will be increased to maintain DO levels ≥75% of saturation. The dilution water may be aerated prior to introduction into the diluter to raise the dissolved oxygen concentration to the maximum achievable level. However, test solutions are not aerated.

11. Lighting - A constant 16-hour light and 8-hour dark photoperiod with a light intensity of 30 to 100 footcandles at the test solution surface is maintained throughout the test. Fluorescent bulbs are used to provide a wide spectrum of light.

CHEMICAL SYSTEM

1. Test Material - Upon arrival at Springborn Laboratories, Inc., the external packaging of the test material is inspected for damage. The packaging is removed and the primary storage container is also inspected for leakage or damage. The sample identity is recorded and the material is stored in the dark at approximately 2-4°C in the original shipping container until used. Exposure of the test material to air should be avoided to minimize the potential for oxidation. The test material should be kept in a tightly sealed container and any head space should be purged of air using nitrogen or helium.

2. Toxicant Concentration Selection - Toxicant concentrations for the partial life cycle test are selected based on information from a 4- to 10-day preliminary flow-through toxicity test with fathead minnow embryos or newly hatched fry. The high concentration in the partial life cycle test is approximately equal to the lowest concentration in the preliminary test causing a significant reduction in survival.

Other available information pertaining to the compound, such as the propensity to bioaccumulate (BCF ≥ 500), might necessitate selecting different toxicant levels. The range of concentrations selected is intended to include both toxicant-effect and non-effect levels; but due to the nature of some compounds, one or both levels may not be observed.

3. Stock Preparation - The stock solution is prepared according to the following formula:

$$\text{Stock concentration} = \frac{\text{H.C. x M.C.}}{\text{B.D. x (\% A.I.} \div 100)}$$

H.C. = high concentration (mg/L)

M.C. = mixing chamber volume (L)

B.D. = bird or syringe delivery (mL)

A.I. = % active ingredient

Test material is weighed on an analytical balance for which a calibration log is maintained. A Chemical Usage Log is also maintained in which the amount, the date, the intended use and the user's initials are recorded each time the test material is used. The stock solution is introduced into the diluter and test aquaria for a minimum of 72 hours before the test is begun, to allow the test solution time to reach equilibrium in the test aquaria.

4. Carrier Solvent - The test material stock solutions are prepared in dilution water without the use of a solvent (carrier).

5. Sampling and Measurements of Toxicant Concentrations - The concentration of test substance will be measured only in the diluter stock solution. Triplicate samples of the stock solution and a single sample of a reagent blank are taken at least twice prior to the initiation of the definitive test, at the initiation of the test (day 0), at hatch and weekly thereafter for determination of toxicant concentration. Three quality control samples are prepared at each sampling interval and remain with the set of samples through extraction, storage and analysis. These samples are prepared in diluent water at test material concentrations similar to the stock concentration. Results of these analyses are indicative of the relative accuracy of the analytical methodologies for each sampling period. Samples are extracted immediately after sampling.

6. Analytical Method-Sample and Stock Stability Studies - The analytical method for the test substance shall be validated prior to beginning the study. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

Prior to initiating the study, the stability of the toxicant stock solution is established. A stock solution consisting of the same concentration of test material and solvent to be used in the

study is prepared. Two aliquots of the stock are removed and analyzed immediately. The stock solution is retained for a minimum of one week under the same conditions as the diluter stock solution (e.g., ambient temperature, laboratory light); then two additional aliquots are removed and analyzed.

7. Measurement of Water Quality Parameters in Exposure Solutions. - At test initiation and weekly thereafter, total hardness, alkalinity, acidity, specific conductance, TOC and particulate matter are measured and recorded in one replicate vessel of the high and low test concentrations and the control. Unionized ammonia will be measured in one replicate control vessel twice each week. Replicates are alternated. Temperature, pH, and dissolved oxygen are recorded in each concentration and control vessel on a daily basis. In addition, a minimum-maximum thermometer is maintained in one of the test solutions, and recorded hourly.

BIOLOGICAL METHODS

1. Embryo Exposure - Fathead minnow eggs used are obtained from the brood unit facility of Springborn Laboratories, Inc. Tests are initiated with eggs that are <24 hours old. Eggs available for initiating a test (minimum of 850) are combined in a Carolina dish filled with 25°C diluent water from the brood unit and placed in a container of 25°C water. Egg cups are placed in a separate container of control water. The water temperature in each bath is maintained at 25 ± 2°C. Eggs are distributed, five at a time, by stratified random assignment to each of the 14 labeled egg cups using a serological pipet. This process is repeated until each cup contains 40 eggs.

   For the next 1-3 days until hatching has begun, each egg cup is examined according to standard operating procedures. The number of live, dead and unaccounted for eggs is recorded daily and the dead eggs are discarded. After hatching has begun, the egg cups are not handled in order to avoid possible physical damage to the newly hatched fry. Only dead eggs and fry are accounted for and removed at this time. Hatching is deemed complete if at the time of observation there are no more than five unhatched eggs remaining in any egg cup. If the number of eggs exceeds five, egg exposure will continue an additional day. When hatch is complete, the number of live, deformed, dead and unaccounted for fry is recorded from each egg cup. Percentage hatch is calculated as the number of live, normal fry in each egg cup/40 eggs. If there were two or more unaccounted for eggs after the first day of egg exposure, then the actual number of eggs (≤ 38) is used as the denominator when computing % hatch. The range of time-to-hatch (to the nearest day) for each embryo incubation cup shall be recorded.

2. Fry through Juvenile Exposure - When hatch is designated as being complete, all surviving larvae are released into the respective test aquaria. If necessary, fry can be transferred from one replicate to the other replicate within a test concentration to achieve equal numbers in each replicate chamber. The first feeding for the fathead minnow fry shall begin shortly after transfer of the fry from the embryo incubation cup to the test chamber. The fry are fed live brine shrimp nauplii (*Artemia salina*) at least three times per day. Routine analysis are conducted on the food source to insure the absence of contamination which would be expected to alter the results of the study. For the first seven days, feeding shall be done at

minimum intervals of four hours (i.e., 8:30 a.m., 12:30 p.m. and 4:30 p.m.). Thereafter, for the duration of the test, the fry shall be fed at approximately 4 hour intervals three times per day on weekdays and twice daily on weekends. Fry shall not be fed 24 hours prior to the termination of the test. Daily observations are recorded on fry mortality, behavior, and appearance. If mortality is obviously occurring in any of the test aquaria, a thorough search for dead fry is made daily in those aquaria. All physical abnormalities (e.g., stunted bodies, scoliosis, etc.) shall be photographed and the deformed fish which die, or are sacrificed at the termination of the test, shall be preserved for possible future pathological examination. Autolyzed fish will be noted as such and discarded. The number of live fry in each aquarium is estimated each day throughout the test. These counts are only estimations due to the difficulty in observing the very small, mobile fry. Average fry survivability must be ≥80% in the controls, and the survival in any control chamber must be >70%.

At the end of the 28-day post hatch exposure period, the fry from each aquarium are anesthetized with MS-222 (tricain methane-sulfonate) and percentage survival, mean total lengths and wet weights are determined for each replicate aquarium. The fry are measured and weighed individually to the nearest mm and mg respectively, to calculate means and standard deviations. The coefficient of variation (100 times the standard deviation divided by the mean) of weights of surviving control fish in each replicate aquarium must not be >40% in order for the test to be acceptable.

An early life stage toxicity test is not acceptable unless at least one of the following criteria is significantly different (p=0.05) from control organisms when compared with treated organisms, and the responses are concentration -dependent: mortality of embryos, hatching success, mortality of fry (at swim-up for trout), total mortality throughout the test, and growth (i.e., weight). If no significant effects occur, but the concentrations tested were the highest possible due to solubility or physio-chemical limitiations, the data will be considered by the Agency for acceptance.

STATISTICS

The endpoints used for determination of significant effect by statistical evaluation include the embryo hatching success, survival of larval or juvenile fish, total length and wet weight. Test data to be statistically analyzed are:

1) Percentage of healthy, fertile embryos at 40-48 hours after the initiation of the test. Percentage is based on the initial number used.

2) Percentage of embryos that produce live fry for release into test chambers. Percentage is based on the number of embryos remaining after thinning.

3) Percentage of embryos that produce live normal, fry for release into test chambers. Percentage is based on the number of embryos remaining after thinning.

4) Percentage of embryos that produce live fish at the end of the test. Percentage is based on the number of embryos remaining after thinning.

5) Percentage of embryos that produce live, normal fish at the end of the test. Percentage is based on the number of embryos remaining after thinning.

6) Individual weight and length of surviving fish at the termination of the test.

The method used to evaluate the results of the early life stage fathead minnow test is *Williams' Test* (Williams, 1971, 1972), coupled with *Bartlett's test* for determination of homogeneity of variances. If necessary, mean values are transformed using square root, arcsine square root, or log conversion procedures. If, after appropriate transformation procedures have been applied to the data, *Bartlett's test* still fails to demonstrate homogeneity of variances, then a non-parametric method is used to compare sample means, such as the *Kruskal-Wallis Test.*

The maximum concentration at which a test material can be present and not be toxic to the test organism is expressed as the Maximum Allowable Toxicant Concentration (MATC). The MATC is determined by taking the geometric mean of the limits set by the lowest test concentration that shows a statistically significant effect (Lowest Observed Effect Concentration, LOEC) and the highest test concentration that shows no statistically significant difference from the control (No Observed Effect Concentration, NOEC).

Transformation of data is limited to data representing endpoint estimates obtained as a proportion (e.g., survival and hatching success). Prior to analyzing data of this type, the observed proportion in each tank is transformed by using the arcsine square-root transformation.

Mortality data for the embryonic stage, fry stages and both stages in replicate exposure chambers will first be analyzed to determine if replicates are significantly different from each other. An example of the statistical analysis would be a two-way analysis of variance (ANOVA) with interaction model. If a significant difference between replicates or a significant interaction exists, cause for the difference should be determined.

REPORTING

All values are reported to various levels of significant depending on the accuracy of the measuring devices employed during any one process. The raw data and final draft of the report are reviewed by the Quality Assurance Unit. Reviewers, other than Quality Assurance, include the Principal Investigator and Study Director. After the final draft has been approved by the above individuals, one copy is sent to the Sponsor. Following review and incorporation of Sponsor's comments, three copies of the final report are issued to the Sponsor. The report will include, but not be limited to, the following information:

* Springborn Laboratories, Inc. report and project numbers.
* Identification of Study Sponsor.
* Laboratory and site, the dates of testing and a list of the personnel involved in the study, i.e., Study Director, Principal Investigator and technicians.

* All information pertaining the test material which appears on the sample bottle, e.g., its source and percent activity, physical properties, Sponsor test material I.D., and sample number.

* Characterization and origin of the dilution water.

* Scientific name of the test organisms, method of verification, source, percent mortality of the adult fish population 48 hours prior to testing, culturing information, and acclimation temperature, pH, and DO range.

* A description of the experimental design, the test chambers and depth and volume of the solution in the chambers, the flow rate as volume addition per 24 hours, the procedure for test initiation, the number of organisms per treatment, the number of replicate chambers per treatment, the biomass loading rate, light intensity and photoperiod and a description of the test substance delivery system.

* Detailed information on feeding of fish during the toxicity test, including type of food used, its source, feeding frequency and results of analysis (i.e., concentration) for contaminants.

* Tabular presentation of all measured and calculated endpoints, as well as definition and/or citation of criteria used to determine the toxic effects and general observations on other effects.

* Description of stock preparation.

* Ranges of water quality variables during the test.

* Results of analytical measurements of stock solutions, and reagent blanks. A detailed description of the analytical procedure(s) used will be provided as an appendix.

* MATC values and the NOEC and LOEC values will be provided where possible, as well as the statistical procedures used to establish these values. These calculations will be made using the nominal test concentrations.

* Reference to the location where raw data are stored.

* Deviations from the protocol not addressed in protocol amendments will be listed, together with a discussion of the impact on the study and signed by the Study Director.

* Good Laboratory Practice (GLP) compliance statement signed by the Study Director.

* Dates of Quality Assurance Audits, signed by the QA Unit.

SPECIAL PROVISIONS

GOOD LABORATORY PRACTICE STANDARDS (GLP): All test procedures, documentation, records, and reports will comply with U.S. Environmental Protection Agency's Good Laboratory Practices Standards, as promulgated under the Toxic Substances Control Act, Part 792 (FEDERAL REGISTER, Part III, 17 August, 1989).

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

TEST MATERIAL ARCHIVAL: It will be the responsibility of the Sponsor to retain a reserve sample of each batch of the test substance, as required by EPA GLP (US EPA, 1983) for studies of greater than 4 weeks duration. Aliquots of the test material can be archived at Springborn Laboratories, Inc. upon request for an additional charge.

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US EPA. 1981. Recommended bioassay procedures for fathead minnows (*Pimephales promelas*) chronic tests. Bioassay Committee of the National Water Quality Laboratory, EPA/ERL Duluth, MN.

U.S. Environmental Protection Agency. 1985, 1987. *Toxic Substances Control Act Test Guidelines.* Federal Register 50(188), September 27, 1985. Amended, May, 1987. "§ 797.1600 Fish Early Life Stage Toxicity Test".

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27: 103-117.

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APPENDIX I

TESTING CONSENT ORDER, CROTONALDEHYDE
(DOCKET# OPTS 42108)

SECTION 797.1660 FATHEAD MINNOW EARLY LIFE STAGE TOXICITY TEST

Section 797.1660 Fish [FATHEAD MINNOW] early life stage toxicity test.

(a) Purpose. This guideline is intended to be used for assessing the propensity of chemical substances to produce adverse effects to fish during the early stages of their growth and development. This guideline describes the conditions and procedures for the continuous exposure of {~~several representative species~~ [FATHEAD MINNOW]} to a chemical substance during egg, fry and early juvenile life stages. The Environmental Protection Agency (EPA) will use data from this test in assessing the potential hazard of the test substance to the aquatic environment.

(b) Definitions. The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in Part 792—Good Laboratory Practice Standards, apply to this section. In addition, the following definitions are applicable to this specific test guideline:

(1) "Acclimation" physiological or behavioral adaptation of organisms to one or more environmental conditions associated with the test method (e.g., temperature, hardness, pH).

(2) "Carrier" solvent or other agent used to dissolve or improve the solubility of the test substance in dilution water.

(3) "Conditioning" exposure of construction materials, test chambers, and testing apparatus to dilution water or to the test solution prior to the start of the test in order to minimize the sorption of test substance onto the test facilities or the leaching of substances from test facilities into the dilution water or the test solution.

(4) "Control" an exposure of test organisms to dilution water only or dilution water containing the test solvent or carrier (no toxic agent is intentionally or inadvertently added).

(5) "Dilution water" the water used to produce the flow-through conditions of the test to which the test substance is added and to which the test species is exposed.

(6) "Early life stage toxicity test" a test to determine the minimum concentration of a substance which produces a statistically significant observable effect on hatching, survival, development and/or growth of a fish species continuously exposed during the period of their early development.

(7) "Embryo cup" a small glass jar or similar container with a screened bottom in which the embryos of some species (i.e., minnow) are placed during the incubation period and which is normally oscillated to ensure a flow of water through the cup.

(8) "Flow through" refers to the continuous or very frequent passage of fresh test solution through a test chamber with no recycling.

(9) "Hardness" the total concentration of the calcium and magnesium ions in water expressed as calcium carbonate (mg $CaCO_3$/liter).

(10) "Loading" the ratio of biomass (grams of fish, wet weight) to the volume (liters) of test solution passing through the test chamber during a specific interval (normally a 24-hr. period).

(11) "No observed effect concentration (NOEC)" the highest tested concentration in an acceptable early life stage test: (i) which did not cause the occurrence of any specified adverse effect (statistically different from the control at the 95 percent level); and (ii) below which no tested concentration caused such an occurrence.

(12) "Observed effect concentration (OEC)" the lowest tested concentration in an acceptable early life stage test: (i) which caused the occurrence of any specified adverse effect (statistically different from the control at the 95 percent level); and (ii) above which all tested concentrations caused such an occurrence.

(13) "Replicate" two or more duplicate tests, samples, organisms, concentrations, or exposure chambers.

(14) "Stock solution" the source of the test solution prepared by dissolving the test substance in dilution water or a carrier which is then added to dilution water at a specified, selected concentration by means of the test substance delivery system.

(15) "Test chamber" the individual containers in which test organisms are maintained during exposure to test solution.

(16) "Test solution" dilution water with a test substance dissolved ~~(or suspended)~~ in it.

(17) "Test substance" the specific form of a chemical substance or mixture that is used to develop data.

(c) Test Procedures—(1) Summary of test. (i) The early life stage toxicity test with fish involves exposure of newly fertilized embryos to various concentrations of a test substance. Exposure continues for 28 days post hatch for the minnows ~~and 60 days post hatch for the trout species.~~ During this time various observations and measurements are made in a specific manner and schedule in order to determine the lowest effect and highest no-effect concentrations of the test substance.

(ii) A minimum of five exposure (treatment) concentrations of a test substance and one control are required to conduct an early life stage toxicity test. The concentration of the test substance in each treatment is usually 50 percent of that in the next higher treatment level.

(iii) For each exposure concentration of the test substance and for each control (i.e., regular control and carrier control is required) there shall be:

(A) At least two replicate test chambers, each containing one or more embryo incubation trays or cups; and there shall be no water connections between the replicate test chambers;

(B) At least 60 embryos divided equally in such a manner that test results show no significant bias from the distributions, between the embryo incubation trays or cups for each test concentration and control (i.e., 30 per embryo cup with 2 replicates);

(C) All surviving larvae divided equally between the test chambers for each test concentration and control (e.g., 30 larvae per test chamber with 2 replicates).

(iv) Duration. (A) For fathead minnow ~~and sheepshead minnow~~ a test begins when the newly fertilized minnow embryos (less than 48-hours old) are placed in the embryo cups and are exposed to the test solution concentrations. The test terminates following 28 days of post-hatch exposure, i.e., 28 days after the newly hatched fry are transferred from the embryo cups into the test chambers.

~~(B) For brook trout and rainbow trout a test begins when newly fertilized trout embryos (less than 96 hours old) are placed in the embryo trays or cups and are exposed to the test solution concentrations. The test terminates following 60 days of post-hatch exposure (for an approximate total exposure period of 90 days).~~

~~(C) For silverside a test begins with newly fertilized embryos (less than or equal to 48 hours old) and is terminated 28 days after hatching. The chorionic fibrils should be cut before randomly placing the embryos in the egg incubation cups.~~

(2) (Reserved)

(3) Range-finding test. (i) A range-finding test is normally performed with the test substance to determine the test concentrations to be used in the early life stage toxicity test, especially when the toxicity is unknown. It is recommended that the test substance concentrations be selected based on information gained from a 4- to 10-day flow-through toxicity test with juveniles of the selected test species.

(ii) The highest concentration selected for the early life stage toxicity test should approximate the lowest concentration indicated in any previous testing to cause a significant reduction in survival. The range of concentrations selected is expected to include both observed effect and no-observed effect levels. The dilution factor between concentrations is normally 0.50, however, other dilution factors may be used as necessary.

(4) Definitive test—(i) General. (A) A test shall not be initiated until after the test conditions have been met and the test substance delivery system has been observed functioning properly for 48 hours. This includes temperature stability, flow requirements of dilution water, lighting requirements, and the function of strainers and air traps included in the water-supply system, and other conditions as specified previously.

(B) New holding and test facilities should be tested with sensitive organisms (i.e., juvenile test species or daphnids) before use to assure that the facilities or substances possibly leaching from the equipment will not adversely affect the test organisms during an actual test.

(C) Embryos should be acclimated for as long as practical to the test temperature and dilution water prior to the initiation of the test.

(D) When embryos are received from an outside culture source (i.e., rainbow and brook trout) at a temperature at variance with the recommended test temperature they shall be acclimated to the test temperature. When eggs are received, they should be immediately unpacked and the temperature of the surrounding water determined. Sudden temperature changes should be avoided. Acclimation to the appropriate test temperature should be accomplished within a period of six hours, and should incorporate the use of dilution water.

(E) Embryos should be visually inspected prior to placement in the embryo cups or screen trays. All dead embryos shall be discarded. Dead embryos can be discerned by a change in coloration from that of living embryos (e.g., trout embryos turn white when dead). During visual inspection, empty shells, opaque embryos and embryos with fungus or partial shells attached shall be removed and discarded. If less than 50 percent of the eggs to be used appear to be healthy, all embryos in such a lot shall be discarded.

(ii) Embryo incubation procedures. (A) Embryos can be distributed to the embryo cups or screen trays using a pipette with a large bore or a similar apparatus. ~~Newly hatched silverside fry are very sensitive to handling, the egg incubation cups should not be handled at all the~~

~~first 5 days after hatching begins. Just before hatching is expected to begin, the embryos should be transferred to clean incubation cups. Trout embryos can be distributed by using a small container which has been precalibrated to determine the approximate number of embryos it can hold; embryos are measured volumetrically in this manner, and are then poured onto the screen tray (or embryo cup). Trout embryos should be separated on the screen tray so that they are not in contact with each other.~~ A final count will ensure the actual number on the screen tray. After random assignment, the screen trays or embryo cups are placed in the test chambers.

(B) Each day until hatch the embryos are visually examined. Minnow embryos may be examined with the aid of a magnifying viewer. ~~Trout embryos should not be touched. Trout embryos should be maintained in low intensity light or in darkness until one week post hatch, and are usually examined with the aid of a flashlight or under low intensity light.~~ Dead embryos should be removed and discarded. Any embryos which are heavily infected with fungus shall be discarded and shall be subtracted from the initial number of embryos used as a basis for the calculations of percentage hatch.

(C) When embryos begin to hatch they should not be handled.

(iii) Initiation of fry exposure. (A) Forty-eight hours after the first hatch in each treatment level, or when hatching is completed, the live young fish shall be counted and transferred from each embryo cup into the appropriate test chamber. ~~For silverside, all surviving fry are not counted until six days after hatching and are not transferred to embryo cups.~~ All of the normal and abnormal fry shall be gently released into the test chamber by allowing the fry to swim out of each embryo cup; nets shall not be used. ~~The trout embryos incubated on screen trays will hatch out in the test chambers, therefore handling of fish is not necessary.~~

(B) If necessary, fry can be transferred from one replicate embryo cup to the other replicate within a test concentration to achieve equal numbers in each replicate chamber.

(C) The number of live fry, live normal fry, live embryos, dead embryos and unaccounted for embryos for each cup shall be recorded when hatching is deemed complete. Those fry which are visibly (without the use of a dissecting scope or magnifying viewer) lethargic or grossly abnormal (either in swimming behavior or physical appearance) should be counted. Late hatching embryos shall be left in the embryo cups to determine if they will eventually hatch or not. The range of time-to-hatch (to the nearest day) for each cup shall be recorded.

(iv) Time to first feeding. (A) The first feeding for the fathead ~~and sheepshead~~ minnow fry shall begin shortly after transfer of the fry from the embryo cups to the test chambers. ~~Silversides are fed the first day after hatch. Trout species initiate feeding at swim-up. The trout fry shall be fed trout starter mash three times a day ad libitum, with excess food siphoned off daily.~~ The minnow fry shall be fed live newly-hatched brine shrimp nauplii (Artemia salina) at least three times a day.

(B) For the first seven days, feeding shall be done at minimum intervals of four hours (i.e., 8 a.m., 12 noon, and 4 p.m.); thereafter the fry shall be fed as indicated below.

(v) Feeding. (A) The fathead ~~and sheepshead~~ minnow fry shall be fed newly-hatched brine shrimp nauplii for the duration of the test at approximately 4-hour intervals three times a day during the week and twice on the weekend after the first week. ~~Trout fry shall be fed at similar intervals and may receive live brine shrimp nauplii in addition to the trout starter food after the first week. Between days 1 and 8 after first hatching, silverside fry are fed the rotifer, Brachionus plicatilis, three times daily at a concentration of 5,000-10,000 organisms per egg cup (based on 15 fish/cup). From days 9-11, the fry shall be fed approximately 3,500 newly-hatched brine shrimp (Artemia) nauplii and 5,000-10,000 rotifers twice daily. For the remainder of the test, the fish will be fed brine shrimp exclusively. The number of organisms used should be gradually increased to approximately 5,000 nauplii by test day 28.~~

(B) An identical amount of food should be provided to each chamber. Fish should be fed ad libitum for 30 minutes with excess food siphoned off the bottom once daily if necessary.

(C) Fish should not be fed for the last 24 hours prior to termination of the test.

~~{(vi) Carriers. Water should be used in making up the test stock solutions. If carriers other than water are absolutely necessary, the amount used should be the minimum necessary to achieve solution of the test substance. Triethylene glycol and dimethyl formamide are preferred, but ethanol and acetone can be used if necessary. Carrier concentrations selected should be kept constant at all treatment levels.)~~

~~{(vii)~~ [(vi)]} Controls. Every test requires a control that consists of the same dilution water, conditions, procedures, and test organisms from the same group used in the other test chambers, except that none of the test substance is added. ~~{If a carrier (solvent) is used, a separate carrier control is required in addition to the regular control.)~~

~~{The carrier control shall be identical to the regular control except that the highest amount of carrier present in any treatment is added to this control. If the test substance is a mixture, formulation, or commercial product, none of the ingredients is considered a carrier unless an extra amount is used to prepare the stock solution.}~~

{~~(viii)~~ [(vii)]} Randomization. The location of all test chambers and species within the test system shall be randomized. [A RESTRICTED RANDOMIZATION WILL BE USED FOR TEST VESSELS.] A representative sample of the test embryos should be impartially distributed by adding to each cup or screen tray no more than 20 percent of the number of embryos to be placed in each cup or screen tray and repeating the process until each cup or screen tray contains the specified number of embryos. Alternatively, the embryos can be assigned by random assignment of a small group (e.g., 1-5) of embryos to each embryo cup or screen tray, followed by random assignment of a second group of equal number to each cup or tray, which is continued until the appropriate number of embryos are contained in each embryo cup or screen tray. The method of randomization used shall be reported in detail.

{~~(ix)~~ [(viii)]} Observations. During the embryo exposure period observations shall be made to check for mortality. During the exposure period of the fry, observations shall be made to check for mortality and to note the physical appearance and behavior of the young fish. The biological responses are used in combination with physical and chemical data in evaluating the overall lethal and sublethal effects of the test substance. Additional information on the specific methodology for the data obtained during the test procedure are discussed in the following sections.

{~~(x)~~ [(ix)]} Biological data. (A) Death of embryos shall be recorded daily.

(B) When hatching commences, daily records of the number of embryos remaining in each embryo cup are required. This information is necessary to quantify the hatching success. A record of all deformed larvae shall be kept throughout the entire post-hatch exposure. ~~Time to swim up shall be recorded for the trout.~~ Upon transfer of fry from the embryo cups to the test chambers, daily counts of the number of live fish should be made. At a minimum, live fish shall be counted on days 4, 11, 18, 25 and ~~(weekly thereafter for the trout species)~~ finally on termination of the test.

(C) The criteria for death of young fish is usually immobility, especially absence of respiratory movement, and lack of reaction to gentle prodding. Deaths should be recorded daily and dead fish removed when discovered.

(D) Daily and at termination of the test, the number of fish that appear (without the use of a magnifying viewer) to be abnormal in behavior (e.g., swimming erratic or uncoordinated, obviously lethargic, hyperventilating, or over excited, etc.) or in physical appearance (e.g., hemorrhaging, producing excessive mucous, or are discolored, deformed, etc.) shall be recorded and reported in detail.

(E) All physical abnormalities (e.g., stunted bodies, scoliosis, etc.) shall be photographed and the deformed fish which die, or are sacrificed at the termination of the test, shall be preserved for possible future pathological examination. [AUTOLYZED FISH WILL BE NOTED AS SO AND DISCARDED.]

(F) At termination, all surviving fish shall be measured for growth. Standard length measurements should be made directly with a caliper, but may be measured photographically. Measurements shall be made to the nearest millimeter (0.1 mm is desirable). Weight measurements shall also be made for each fish alive at termination (wet, blotted dry and to the nearest 0.01 g for the minnows ~~and 0.1 g for the trout~~). If the fish exposed to the toxicant appear to be edematous compared to control fish, determination of dry, rather than wet, weight is recommended.

(G) Special physiological, biochemical and histological investigations on embryos, fry, and juveniles may be deemed appropriate and shall be performed on a case by case basis. [TEST ORGANISM SPECIMENS WILL BE COLLECTED AND PRESERVED DURING THE TEST AT THE DISCRETION OF THE STUDY DIRECTOR.]

(5) Test results. ~~(i)~~ Data from toxicity tests are usually either continuous (e.g., length or weight measurements) or dichotomous (e.g., number hatching or surviving) in nature. Several methods are available and acceptable for statistical analysis of data derived from early life stage toxicity tests; however, the actual statistical methodology to analyze and interpret the test results shall be reported in detail.

(ii) The significance level for all statistical testing shall be a minimum of P=0.05 (95 percent confidence level).

(A) Example of statistical analysis. (1) Mortality data for the embryonic stage, fry stage and for both stages in replicate exposure chambers should first be analyzed using a two-way analysis of variance (ANOVA) with interaction model. This analysis will determine if replicates are significantly different from each other. If a significant difference between replicates or a significant interaction exists, cause for the difference should be determined.

Modification should then be made in the test apparatus or in handling procedures for future toxicity tests. Further calculations should incorporate the separation of replicates. If no significant difference is observed, replicates may be pooled in further analyses.

(2) After consideration of replicate responses, mortality data should then be subjected to one-way ANOVA. The purpose of this analysis is to determine if a significant difference exists in the percentage mortality between control fish and those exposed to the test material.

(3) If the one-way ANOVA results in a F ratio that is significant, it would be acceptable to perform t-tests on the control versus each concentration. A second technique is to identify treatment means that are significantly different; this method should involve the additional assumption that the true mean response decreases generally with increasing concentration. The researcher may also be interested in determining significant differences between concentrations.

(4) Growth data should also be analyzed by one-way ANOVA with the inclusion of a covariate to account for possible differences in growth of surviving fry in embryo cup(s) that contain fewer individuals. This condition can occur in cases when the same amount of food is given to each test chamber regardless of the number of survivors.

(B) Test data to be analyzed. Data to be statistically analyzed are:

(1) Percentage of healthy, fertile embryos at 40-48 hours after initiation of the test. Percentage is based upon initial number used.

(2) Percentage of embryos that produce live fry for release into test chambers. Percentage is based on number of embryos remaining after thinning.

(3) Percentage of embryos that produce live, normal fry for release into test chambers. Percentage is based upon number of embryos remaining after thinning.

~~(4) Percentage of fry survival at swim-up for trout. Percentage is based upon number of embryos remaining after thinning.~~

{~~(5)~~ [(4)]} Percentage of embryos that produce live fish at end of test. Percentage is based upon number of embryos remaining after thinning.

{~~(6)~~ [(5)]} Percentage of embryos that produce live, normal fish at end of test. Percentage is based upon number of embryos remaining after thinning.

{~~(7)~~ [(6)]} Weights and lengths of individual fish alive at the end of the test.

(C) It is important that fish length and weight measurements be associated with individual test chambers since the density of the fish and available food should be considered in the growth of the organism.

(iii) Acceptability criteria. (A) An early life stage toxicity test is not acceptable unless at least one of the following criteria is significantly different (p=0.05) from control organisms when compared with treated organisms, and the responses are concentration-dependent: mortality of embryos, hatching success, mortality of fry (at swim-up for trout), total mortality throughout the test, and growth (i.e., weight). If no significant effects occur, but the concentrations tested were the highest possible due to solubility or other physio-chemical limitations, the data will be considered for acceptance.

(B) In addition to obtaining significant effects on the exposed test species, a measure of acceptability in the response of control fish is also required.

(C) A test is not acceptable if the average survival of the control fish at the end of the test is less than 80 percent or if survival in any one control chamber is less than 70 percent. ~~For silversides, a test is not acceptable if the average overall survival of the control embryos and fish at the end of the test is less than 60 percent.~~

~~{(D) If a carrier is used, the criteria for effect (mortality of embryos and fry, growth, etc.) used in the comparison of control and exposed test organisms shall also be applied to the control and control with carrier chambers. For the test to be considered acceptable, no significant difference shall exist between these criteria.}~~

{~~(E)~~ [(D)]} A test is not acceptable if the relative standard deviation (RSD=100 times the standard deviation divided by the mean) of the weights of the fish that were alive at the end of the test in any control test chamber is greater than 40 percent.

(6) Analytical measurements—(i) Analysis of water quality. Measurement of certain dilution water quality parameters shall be performed every 6 months, to determine the consistency of the dilution water quality. In addition, if data in 30 day increments are not available to show that freshwater dilution water is constant, measurements of hardness, alkalinity, pH, acidity, conductivity, TOC or COD and particulate matter should be conducted once a week in the highest test substance concentration. Measurement of calcium, magnesium, sodium, potassium, chloride, and sulfate is desirable. [FOR THE ANALYTICAL REQUIREMENTS OF THE DILUENT WATER, THE ATTACHED DILUTION WATER AGGREGATE HISTORICAL DATA SUMMARY WILL BE SUBSTITUTED. THE MONDAY AND THURSDAY MEASURED RESIDUAL CHLORINE SHOULD BE LESS THAN 0.01 mg/l.]

(ii) Dissolved oxygen measurement. The dissolved oxygen concentration shall be measured in each test chamber at the beginning of the test and at least once weekly thereafter (as long as live organisms are present) in two replicates of the control and the high, medium, and low test substance concentrations.

(iii) Temperature measurement. Temperatures shall be recorded in all test chambers at the beginning of the test, once weekly thereafter and at least hourly in one test chamber. When possible, the hourly measurement shall be alternated between test chambers and between replicates.

(iv) Test substance measurement. (A) Prior to the addition of the test substance to the dilution water, {~~it is recommended that~~} the test substance stock solution {[WILL]} be analyzed to verify the concentration. {~~After addition of the test substance, the concentration of test substance should be measured at the beginning of the test in each test concentration and control(s), and in one replicate at each test concentration at least once a week thereafter. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. If a malfunction in the delivery system is discovered, water samples shall be taken from the affected test chambers immediately and analyzed.~~}

{~~(B) The measured concentration of test substance in any chamber should be no more than 20 percent higher or lower than the concentration calculated from the composition of the stock solution and the calibration of the test substance delivery system. If the difference is more than 20 percent, the concentration of test substance in the solution flowing into the exposure chamber (influent) should be analyzed. These results will indicate whether the problem is in the stock solution, the test substance delivery system or in the test chamber. Measurement of degradation products of the test substance is recommended if a reduction of the test substance concentration occurs in the test chamber.~~}

-12- [797.1660 FM]

(v) Sampling and analysis methodology. ~~(A) Generally, total test substance measurements are sufficient; however, the chemical characteristics of the test substance may require both dissolved and suspended test substance measurements.~~

[(A) {~~DEIONIZED OR DISTILLED WATER SHOULD BE USED IN MAKING STOCK SOLUTIONS OF THE TEST SUBSTANCE.~~} STANDARD ANALYTICAL METHODS SHOULD BE USED WHENEVER AVAILABLE IN PERFORMING THE ANALYSES. THE ANALYTICAL METHOD USED TO MEASURE THE AMOUNT OF TEST SUBSTANCE IN A SAMPLE SHALL BE VALIDATED BEFORE BEGINNING THE TEST BY APPROPRIATED LABORATORY PRACTICES. AN ANALYTICAL METHOD IS NOT ACCEPTABLE IF LIKELY DEGRADATION PRODUCTS OF THE TEST SUBSTANCE, SUCH AS HYDROLYSIS AND OXIDATION PRODUCTS, GIVE POSITIVE OR NEGATIVE INTERFERENCES WHICH CANNOT BE SYSTEMATICALLY IDENTIFIED AND CORRECTED MATHEMATICALLY.]

[(B) THE ANALYTICAL METHOD FOR THE TEST SUBSTANCE SHALL BE VALIDATED PRIOR TO BEGINNING THE TEST. A PROCEDURE SUCH AS USING KNOWN ADDITIONS MAY BE USED. THIS INVOLVES ADDING {[A]} KNOWN AMOUNT{~~S~~} OF THE TEST SUBSTANCE TO THREE OR MORE SAMPLES OF DILUTION WATER. THE NOMINAL CONCENTRATION{~~S~~} OF THE TEST SUBSTANCE IN THESE SAMPLES SHOULD {~~EQUAL~~ [APPROXIMATE]} THE CONCENTRATION {~~RANGE~~ [IN THE STOCK SOLUTION]} TO BE USED {~~IN THE TEST~~}. {~~BOTH DISSOLVED TEST SUBSTANCE (THAT WHICH PASSES THROUGH A 0.45 MICRON FILTER) AND TOTAL TEST SUBSTANCE SHALL BE MEASURED IN EACH SAMPLE. IF THE MEASURED CONCENTRATIONS OF DISSOLVED TEST SUBSTANCE ARE GREATER THAN 80% OF THE MEASURED CONCENTRATIONS OF TOTAL TEST SUBSTANCE, THEN ONLY TOTAL TEST SUBSTANCE SHALL BE MEASURED DURING THE TEST. HOWEVER, IF THE MEASURED CONCENTRATIONS OF DISSOLVED TEST SUBSTANCE ARE LESS THAN 80% OF THE MEASURED CONCENTRATIONS OF TOTAL TEST SUBSTANCE, THEN ONLY DISSOLVED TEST SUBSTANCE SHALL BE MEASURED DURING THE TEST.~~} VALIDATION OF THE ANALYTICAL METHOD SHOULD BE PERFORMED ON AT LEAST TWO SEPARATE DAYS PRIOR TO STARTING THE TEST.]

[(C) SUBJECT TO CONSTRAINTS ASSOCIATED WITH LIMITS OF DETECTION;. {~~ALL DOSE LEVELS~~ [THE STOCK SOLUTION]} WILL BE ANALYZED FOR THE TEST ARTICLE AT {[THE START OF THE EXPOSURE AND AT]} LEAST ONCE EVERY SEVEN DAYS ([THEREAFTER]). {~~EQUAL ALIQUOTS OF TEST ARTICLE SOLUTION (OR CONTROL SOLUTION) MAY BE REMOVED FROM REPLICATE TEST VESSELS AND COMBINED FOR ANALYSIS.~~} IN ADDITION TO ANALYZING SAMPLES OF TEST SOLUTION, AT LEAST ONE REAGENT BLANK, CONTAINING ALL REAGENTS USED, SHOULD ALSO BE ANALYZED.]

{[~~(D) FILTERS AND THEIR HOLDERS USED FOR DETERMINING THE DISSOLVED TEST SUBSTANCE CONCENTRATIONS SHOULD BE PREWASHED WITH SEVERAL VOLUMES OF DISTILLED WATER OR DILUTION WATER AND UNDERGO A FINAL RINSE WITH TEST SOLUTION. GLASS OR STAINLESS STEEL FILTER HOLDERS ARE BEST FOR ORGANIC SUBSTANCES, WHILE PLASTIC HOLDERS ARE BEST FOR METALS. THE SAMPLE SHOULD BE FILTERED WITHIN 30 MINUTES AFTER IT IS TAKEN FROM THE TEST CHAMBER.~~]}

~~(2)[(E)]~~ [(D)] ~~For measurement of the test substance, water samples shall be taken midway between the top, bottom, and sides of the test chamber and should not include any surface scum or material stirred up from the bottom or sides.~~) Samples of test solutions shall be handled and stored appropriately to minimize loss of test substance by microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

~~(3) Chemical and physical analyses shall be performed using standardized methods whenever possible. The analytical method used to measure the concentration of the test substance in the test solution shall be validated before the beginning of the test. At a minimum, a measure of the accuracy of the method should be obtained on each of two separate days by using the method of known additions, and using dilution water from a test containing test organisms. Three samples should be analyzed at the next to lowest test substance concentration. It is also desirable to study the accuracy and precision of the analytical method for test guideline determination by use of reference (split) samples, or interlaboratory studies, and by comparison with alternative, reference, or corroborative methods of analysis.~~

~~(2) An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative inferences, unless it is shown that such degradation products are not present in the test chambers during the test. In general, atomic absorption spectrophotometric methods for metals and gas chromatographic methods for organic compounds are preferable to colorimetric methods.~~

~~(3) In addition to analyzing samples of test solution, at least one reagent blank also should be analyzed when a reagent is used in the analysis. Also, at least one sample for the method of known additions should be prepared by adding test substance at the concentration used in the toxicity test.~~

(d) Test conditions—(1) Test species. (i) One or more of the recommended test species will be specified in rules under Part 799 in this chapter requiring testing of specific chemicals. The recommended test species are:

(A) Fathead minnow (Pimephales promelas Rafinesque). [THIS WILL BE THE TEST SPECIES FOR THIS PROTOCOL.]

~~(B) Sheepshead minnow (Cyprinodon variegatus).~~

~~(C) Brook trout (Salvelinus fontinalis).~~

~~(D) Rainbow trout (Salmo gairdneri).~~

~~(E) Atlantic silverside (Menidia menidia).~~

~~(F) Tidewater silverside (Menidia peninsulae).~~

ii) Embryos used to initiate the early life stage test shall be less than 48 hours old for the fathead ~~and sheepshead~~ minnows, ~~silversides, and less than 96 hours old for the brook trout and rainbow trout~~. In addition, the following requirements shall be met:

(A) All embryos used in the test shall be from the same source. Embryos shall be obtained from a stock cultured in-house when possible, and maintained under the same parameters as specified for the test conditions. When it is necessary to obtain embryos from an external source, caution should be exercised to ensure embryo viability and to minimize the possibility of fungal growth. A description of the brood stock history or embryo source shall be made available to EPA upon request.

(B) Test species shall be cared for and handled properly in order to avoid unnecessary stress. To maintain test species in good condition and to maximize growth, crowding shall be prevented, and the dissolved oxygen level shall be maintained near saturation.

(C) Embryos and fish shall be handled as little as possible. Embryos shall be counted and periodically inspected until hatching begins. When larvae begin to hatch, they shall not be handled. Transfer of minnow larvae from embryo cups to test chambers shall not involve the use of nets. No handling is necessary following introduction into the test chambers until termination of the test.

(D) If fathead minnow embryos are obtained from in-house culture units, the embryos should be gently removed from the spawning substrate. The method for separating the fertilized eggs from the substrate is important and can affect the viability of the embryos; therefore the finger-rolling procedure is recommended.

(E) Disease treatment. Chemical treatments to cure or prevent diseases should not be used before, and should not be used during a test. All prior treatments of brood stock should be reported in detail. Severely diseased organisms should be destroyed.

(2) Test facilities—(i) Construction materials. Construction materials and equipment that contact stock solutions, test solutions, or dilution water into which test embryos or fish are placed should not contain any substances that can be leached or dissolved into aqueous solutions in quantities that can affect test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test chemicals from dilution water. Glass, #316 stainless steel, nylon

screen and perfluorocarbon plastic (e.g., Teflon©) are acceptable materials. Concrete or rigid (unplasticized) plastic may be used for holding and acclimation tanks, and for water supply systems, but they should be thoroughly conditioned before use. If cast iron pipe is used in freshwater supply systems, colloidal iron may leach into the dilution water and strainers should be used to remove rust particles. Natural rubber, copper, brass, galvanized metal, epoxy glues, and flexible tubing should not come in contact with dilution water, stock solutions, or test solutions.

(ii) Test chambers (exposure chambers). (A) Stainless steel test chambers should be welded or glued with silicone adhesive, and not soldered. Glass should be fused or bonded using clear silicone adhesive. Epoxy glues are not recommended, but if used ample curing time should be allowed prior to use. As little adhesive as possible should be in contact with the water.

(B) Many different sizes of test chambers have been used successfully. The size, shape and depth of the test chamber is acceptable if the specified flow rate and loading requirements can be achieved.

(C) The actual arrangement of the test chambers can be important to the statistical analysis of the test data. Test chambers can be arranged totally on one level (tier) side by side, or on two levels with each level having one of the replicate test substance concentrations or controls. Regardless of the arrangement, it shall be reported in detail and considered in the data analysis. [RESTRICTED RANDOMIZATION WILL BE USED FOR TEST VESSEL PLACEMENT.]

(iii) Embryo incubation apparatus. ~~(A) Recommended embryo incubation apparatus include embryo cups for the minnow species and screen trays for the trout species, although embryo cups can be used for the trout species. Embryo cups are normally constructed from approximately 4-5 cm inside diameter, 7-8 cm high glass jars with the end cut off or similar sized sections of polyethylene tubing. One end of the jar or tubing is covered with stainless steel or nylon screen (approximately 40 meshes per inch is recommended).~~

~~Embryo cups for silversides are normally constructed by using silicone adhesive to glue a 10 cm high, 363 um nylon mesh tube inside a 9 cm I.D. glass Petri dish bottom. The embryo cups shall be appropriately labeled and then suspended in the test chamber in such a manner as to ensure that the test solution regularly flows through the cup and that the embryos are always submerged but are not agitated too vigorously. Cups may be oscillated by a rocker arm apparatus with a low rpm motor (e.g., 2 rpm) to maintain the required flow of test water. The vertical travel distance of the rocker arm apparatus during oscillation is normally 2 1/2 - 3 cm. The water level in the test chambers may also be varied by means of a self-starting siphon in order to ensure exchange of water in the embryo cups.~~

~~(B) The trout embryo incubation trays can be made from stainless steel screen (or other acceptable material such as plastic) of about 2.4 mm mesh. The screen tray should be supported above the bottom of the test chamber by two folds of screen or other devices which function as legs or supports. The edges of the screen tray should be turned up to prevent bump spills and to prevent the embryos from rolling off in the event of excessive turbulence. Suspending or supporting the screen tray off the bottom ensures adequate water circulation around the embryos and avoids contact of embryos with possible bottom debris.~~ [PARTITIONED LEXAN FLOW-THROUGH HATCHING BOATS WILL BE USED FOR THE EMBRYO INCUBATION APPARATUS.]

(iv) Test substance delivery system. (A) The choice of a specific delivery system depends upon the specific properties and requirements of the test substance. The apparatus used should accurately and precisely deliver the appropriate amount of stock solution and dilution water to the test chambers. The system selected shall be calibrated before each test. Calibration includes determining the flow rate through each chamber, and the proportion of stock solution to dilution water delivered to each chamber. The general operation of the test substance delivery system shall be checked at least twice daily for normal operation throughout the test. A minimum of five test substance concentrations and one control shall be used for each test.

(B) The proportional diluter and modified proportional diluter systems and metering pump systems have proven suitable and have received extensive use.

(C) Mixing chambers shall be used between the diluter and the test chamber(s). This may be a small container or flow-splitting chamber to promote mixing of test substance stock solution and dilution water, and is positioned between the diluter and the test chambers for each concentration. If a proportional diluter is used, separate delivery tubes shall run from the flow-splitting chamber to each replicate test chamber. Daily checks on this latter system shall be made.

~~(D) Silverside fry are injured easily and are susceptible to impingement to the mesh of the incubation cups. Consequently, water flow into and out of the cups when counting fry must be at a slow rate. This can be accomplished by using small diameter (e.g., 2 mm I.D.) capillary tubes to drain the test solution from splitter boxes into the replicate test chambers. The use of a self-starting siphon to gradually lower (i.e., less than or equal to 1 min.) the water level approximately 2 cm in the test chamber is recommended. A minimum water depth of 5 cm should be maintained in the cups. Although it may be satisfactory, a rocker arm type apparatus has not yet been used with silversides.~~

(v) Other equipment required. (A) An apparatus for removing undesirable organisms, particulate matter and air bubbles.

(B) An apparatus for aerating water.

(C) A suitable magnifying viewer for examination of minnow embryos.

(D) A suitable apparatus for the precise measurement of growth of the fish, including both length (e.g., with metric or ruler caliper or photographic equipment) and weight.

(E) Facilities for providing a continuous supply of live brine shrimp nauplii (Artemia salina).

~~(F) For silversides, facilities for providing a supply of rotifers (Brachionus plicatilis) for approximately 11 days.~~

~~(G)~~ [(F)]] Facilities (or access to facilities) for performing the required water chemistry analyses.

(vi) Cleaning of equipment. (A) Test substance delivery systems and test chambers should be cleaned before use. Test chambers should be cleaned during the test as needed to maintain the dissolved oxygen concentration, and to prevent clogging of the embryo cup screens and narrow flow passages.

(B) Debris can be removed with a rubber bulb and large pipette or by siphoning with a glass tube attached to a flexible hose. Debris should be run into a bucket light enough to observe that no live fish are accidentally discarded.

(vii) Dilution water—(A) General. (1) A constant supply of acceptable dilution water should be available for use throughout the test. Dilution water shall be of a minimum quality such that the test species selected will survive in it for the duration of testing without showing signs of stress (e.g., loss of pigmentation, disorientation, poor response to external stimuli, excessive mucous secretion, lethargy, lack of feeding or other unusual behavior). A better criterion for an acceptable dilution water for tests on early life stages should be such that the species selected for testing will survive, grow and reproduce satisfactorily in it.

(2) The concentration of dissolved oxygen in the dilution water (fresh or salt) shall be between 90 percent and 100 percent saturation. When necessary, dilution water should be aerated by means of airstones, surface aerators, or screen tubes before the introduction of the test substance.

(3) Water that is contaminated with undesirable microorganisms (e.g., fish pathogens) shall not be used. If such contamination is suspected, the water should be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter before use. Efficacy of the sterilizer can be determined by using standard plate count method.

(B) Freshwater. (1) Natural water (clean surface or ground water) is preferred, however, dechlorinated tap water may be used as a last resort. Reconstituted freshwater is not recommended as a practical dilution water for the early life stage toxicity test because of the large volume of water required.

(2) Particulate and dissolved substance concentrations should be measured at least twice a year and should meet the following specifications. [FOR THE ANALYTICAL REQUIREMENTS OF THE DILUENT WATER, THE ATTACHED AGGREGATE HISTORICAL DATA SUMMARY WILL BE SUBSTITUTED. THE MONDAY AND THURSDAY MEASURED RESIDUAL CHLORINE SHOULD BE LESS THAN 0.01 mg/L.]

Substance	Maximum Concentration	
Particulate matter	<20	mg/liter.
Total organic carbon (TOC)	~~<2~~ [3]	mg/liter.
Chemical oxygen demand (COD)	<5	mg/liter.
Un-ionized ammonia	~~<1~~ [20]	µg/liter.
Residual chlorine	~~<1~~ [10]	µg/liter.
Total organophosphorus pesticides	<50	ng/liter.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	<50	ng/liter.
Total organic chlorine	<25	ng/liter.

(3) During any one month, [WEEKLY ANALYSIS OF THE DILUTION WATER SHOULD SHOW THAT THE FOLLOWING CHARATERISTICS DO] ~~freshwater dilution water should~~ not vary more than 10 percent from the respective monthly averages[:] or hardness, alkalinity and specific conductance; the monthly pH range should be less than 0.4 pH units.

~~(C) Saltwater. (1) Marine dilution water is considered to be of constant quality if the minimum salinity is greater than 15 0/00 and the weekly range of the salinity is less than 15 0/00. The monthly range of pH shall be less than 0.8 pH units. Saltwater shall be filtered to remove larval predators. A pore size of < 20 micrometers (um) is recommended. For silversides, the recommended salinity is 20 ppt and shall be maintained between 15 and 25 ppt throughout testing.~~

~~(2) Artificial sea salts may be added to natural seawater during periods of low salinity to maintain salinity above 15 0/00.~~

~~(2)~~ [(C)]] Test parameters—(i) Dissolved oxygen concentration. It is recommended that the dissolved oxygen concentration be maintained between 90 and 100 percent saturation; but it shall be no less than ~~75~~ [60]] percent saturation at all times ~~(for both minnow species) and between 90 and 100 percent saturation for the trout species in all test chambers~~. Dilution water in the head box may be aerated, but the test solution itself shall not be aerated.

(ii) Loading and flow rate. (A) The loading in test chambers should not exceed 0.1 grams of fish per liter of test solution passing through the test chamber in 24 hours. The flow rate to each chamber should be a minimum of 6 tank volumes per 24 hours. During a test, the flow rates should not vary more than 10 percent from any one test chamber to any other.

(B) A lower loading or higher flow rate or both shall be used if necessary to meet the following three criteria at all times during the test in each chamber containing live test organisms:

(1) The concentration of dissolved oxygen ~~shall~~ [SHOULD] not fall below 75 percent saturation [AND SHALL BE AT LEAST 60% SATURATION] for the fathead ~~and cheesehead~~ minnows ~~and 90 percent for the rainbow and brook trout~~;

(2) The concentration of un-ionized ammonia should not exceed ~~1~~[20] µg/l [DETERMINATIONS WILL BE MADE IN THE CONTROLS ON MONDAY AND THURSDAY. ON SITE MEASUREMENT WITH A TEST KIT FOR TOTAL NH_3 AT 0.1 mg/L. UN-IONIZED AMMONIA WILL BE DIRECTLY DETERMINED IN ACCORDANCE TO TEMPERATURE AND pH.]; and

~~(3) The concentration of toxicant should not be lowered (i.e., caused by uptake by the test organisms and/or materials on the sides and bottoms of the chambers) more than 20 percent of the mean measured concentration. [THIS DETERMINATION WILL BE BASED ON THE MEAN OF TEST SOLUTION ANALYSIS CONDUCTED PRIOR TO TEST START.]~~)

(iii) Temperature. (A) The recommended test temperatures are:

(1) Fathead minnow—25°C for all life stages.

~~(2) Cheesehead minnow—20°C for all life stages.~~

~~(3) Rainbow and brook trout—10°C for embryos—12°C for fry and alevins.~~

~~(4) Atlantic and tidewater silversides--25°C for all life stages.~~

(B) Excursions from the test temperature shall be no greater than ± 2.0°C. It is recommended that the test system be equipped with an automatic alarm system to alert staff of instantaneous temperature changes in excess of 2°C. If the water is heated (i.e., for minnow species), precautions should be taken to ensure that supersaturation of dissolved gases is avoided. Temperatures shall be recorded in all test chambers at the beginning of the test and weekly thereafter. The temperature shall be recorded at least hourly in one test chamber throughout the test.

(iv) Light. ~~(A) Brook and rainbow trout embryos shall be maintained in darkness or very low light intensity through one week post hatch, at which time a 14 hour light and 10 hour dark photoperiod shall be provided.~~

{~~(B)~~ [(A)]} For fathead ~~and sheepshead~~ minnows, a 16-hour light and 8-hour dark (or 12:12) photoperiod shall be used throughout the test period.

~~(C) For silversides, a 14 hour light and 10 hour dark photoperiod shall be used throughout the test period.~~

{~~(D)~~ [(B)]} A 15-minute to 30-minute transition period between light and dark is optional.

{~~(E)~~ [(C)]} Light intensities ranging from 30 to 100 lumens at the water surface shall be provided; the intensity selected should be duplicated as closely as possible for all test chambers [I.E., 30 TO 100 FOOT CANDLES].

(e) Reporting. A report of the results of an early life stage toxicity test shall include the following:

(1) Name of test, sponsor, investigator, laboratory, and dates of test duration.

(2) Detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers (solvents) or other additives used.

(3) The source of the dilution water, its chemical characteristics, and a description of any pretreatment.

(4) Detailed information about the test organisms including scientific name and how verified and source history, observed diseases, treatments, acclimation procedure, and concentration of any contaminants and the method of measurement.

(5) A description of the experimental design and the test chambers, the depth and volume of the solution in the chambers, the way the test was begun, the number of organisms per treatment, the number of replicates, the loading, the lighting, a description of the test substance delivery system, and the flow rate as volume additions per 24 hours.

(6) Detailed information on feeding of fish during the toxicity test, including type of food used, its source, feeding frequency and results of analysis (i.e., concentrations) for contaminants. [THE AGENCY WILL SUPPLY THE METHODS FOR FEED ANALYSIS.]

(7) Number of embryos hatched, number of healthy embryos, time to hatch, mortality of embryos and fry, measurements of growth (weight and length), incidence of pathological or histological effects and observations of other effects or clinical signs, number of healthy fish at end of test.

(8) Number of organisms that died or showed an effect in the control and the results of analysis for concentration(s) of any contaminant in the control(s) should mortality occur.

(9) Methods used for, and the results of (with standard deviation), all chemical analyses of ~~water quality and~~ test substance concentration, including validation studies and reagent blanks; the average and range of the test temperature(s). [FOR THE ANALYTICAL REQUIREMENTS OF THE DILUENT WATER, THE ATTACHED AGGREGATE HISTORICAL DATA SUMMARY WILL BE SUBSTITUTED. LIMITS OF DETECTION FOR EACH OF THE ANALYTES SHALL BE INCLUDED.]

(10) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(11) A description of any abnormal effects and the number of fish which were affected during each period between observations in each chamber, and the {~~average~~ [NOMINAL]} concentration of test substance in each test chamber.

(12) Reference to the raw data location.

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APPENDIX II

GFT Grab Water Sample*		
Sample Range: 3/29/89 - 1/16/92		
Pesticide Screen I;II;III	Results Received (Range)	Maximum Limit of Quantitation
Alpha BHC	< 0.01 - < 0.02 µg/L	0.02
Beta BHC	< 0.02 - 0.01 µg/L	0.02
Gamma BHC - Lindane	<0.02 - 0.01 µg/L	0.02
Delta BHC	< 0.01 - < 0.02 µg/L	0.02
Heptachlor	< 0.01 - < 0.02 µg/L	0.02
Aldrin	< 0.01 - < 0.02 µg/L	0.02
Heptachlor Epoxide	< 0.01 - < 0.02 ug/L	0.02
DDE	< 0.01 - < 0.02 µg/L	0.02
DDD	< 0.01 - < 0.02 µg/L	0.02
DDT	< 0.01 - < 0.02 µg/L	0.02
HCB	< 0.01 - < 0.02 µg/L	0.02
Mirex	< 0.01 - < 0.02 µg/L	0.02
Methoxychlor	< 0.05 - < 0.2 µg/L	0.1
Dieldrin	< 0.01 - < 0.02 µg/L	0.02
Endrin	< 0.01 - < 0.02 µg/L	0.02
Telodrin	< 0.01 - < 0.02 µg/L	0.02
Chlordane	< 0.05 - < 0.1 µg/L	0.1
Toxaphene	< 0.1 - < 2. µg/L	2.
PCB's	< 0.2 - < 2. µg/L	2.
Ronnel	< 0.01 - < 0.02 µg/L	0.02
Ethion	< 0.02 - < 0.05 µg/L	0.05
Trithion	< 0.05 - < 0.1 µg/L	0.1
Diazinon	< 0.1 - < 0.5 µg/L	0.5
Methyl Parathion	< 0.02 - < 0.1 µg/L	0.1
Ethyl Parathion	< 0.05 - < 0.1 µg/L	0.1
Malathion	< 0.05 - < 0.2 µg/L	0.2
Endosulfan I	< 0.01 - < 0.02 µg/L	0.02
Endosulfan II	< 0.01 - < 0.02 µg/L	0.02
Endosulfan Sulfate	< 0.03 - < 0.1 µg/L	0.1
* Analyzed by Lancaster Laboratories, Inc.		

GFT Grab Water Sample*		
Sample Range: 3/2/89 - 1/16/92		
ICP Metals, Screen II	Results Received (Range)	Maximum Limit of Quantitation
Pesticide Screen I,II,III	attached	
Mercury	< 0.0005 mg/L	0.0005
Arsenic	< 0.05 mg/L	0.05
Selenium	< 0.05 mg/L	0.05
Boron	< 0.005 - < 0.05 mg/L	0.05
Thallium	< 0.1 mg/L	0.1
Aluminum	< 0.1 - < 0.2 mg/L	0.2
Antimony	< 0.05 mg/L	0.05
Barium	< 0.1 - < 0.2 mg/L	0.2
Beryllium	< 0.005 - 0.005 mg/L	0.005
Cadmium	< 0.005 - < 0.05 mg/L	0.005
Calcium	2.3 - 8.7 mg/L	0.5
Chromium	< 0.05 mg/L	0.05
Cobalt	< 0.05 mg/L	0.05
Copper	< 0.02 - < 0.05 mg/l	0.05
Iron	< 0.05 - 0.1 mg/L	0.1
Lead	< 0.05 mg/L	0.05
Lithium	< 0.5 mg/L	0.5
Magnesium	1.1 - 2.1 mg/L	0.5
Manganese	< 0.01 - 0.03 mg/L	0.01
Molybdenum	< 0.1 mg/L	0.1
Nickel	< 0.04 - < 0.05 mg/L	0.04
Potassium	0.5 - 1.2 mg/L	0.5
Silicon	4.2 - < 5. mg/L	0.5 - 5**
Silver	< 0.01 - < 0.05 mg/L	0.05
Sodium	5.1 - 12.8 mg/L	0.5
Strontium	< 0.05 mg/L	0.05
Titanium	< 0.05 mg/L	0.05
Vanadium	< 0.05 mg/L	0.05
Zinc	< 0.02 - < 0.05 mg/L	0.05
* Analyzed by Lancaster Laboratories, Inc.		
** For 10/30/90 sample, the quantitation limit was increased due to nature of sample matrix		

Springborn Laboratories, Inc.
Environmental Sciences Division
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PROTOCOL AMENDMENT

AMENDMENT #: 1

DATE: 20 August 1992

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with Fathead Minnow, *Pimephales promelas* Following TSCA Guideline 797-1600."

SPECIES: *Pimephales promelas*

STUDY SPONSOR: Eastman Kodak Company

TEST MATERIAL: Crotonaldehyde

SLI STUDY NO: 1852.0692.6102.120

AMENDMENT(S): The protocol states that the test material stock solutions are prepared in dilution water without the use of a solvent (carrier). During this study the test material stock solutions are prepared in ASTM Type II water (purified using a Nanopure® system) due to increased stability in this type of water.

Approval Signatures: Mark W. Machado 8/20/92 DCG
Mark W. Machado — Date
SLI Study Director

Joseph W. Gorsuch 8/21/92
Joseph W. Gorsuch — Date
Sponsor Study Monitor

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

PROTOCOL AMENDMENT

AMENDMENT #: 2

DATE: 03 November 1992

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Life-Cycle Toxicity Test with Fathead Minnow, *Pimephales promelas* Following TSCA Guideline 797-1600."

SPECIES: *Pimephales promelas*

STUDY SPONSOR: Eastman Kodak Company

TEST MATERIAL: Crotonaldehyde

SLI STUDY NO: 1852.0692.6102.120

AMENDMENT(S): The study protocol designates the nominal test concentrations for the definitive study as 2.0, 0.98, 0.49, 0.25, 0.12 and 0.061 mg A.I./L plus a dilution water control. Based on the corrected percent A.I. (Active Ingredient) of the test material (i.e., 93.8% Crotonaldehyde) and on the corrected mixing chamber volume determined through the weekly calibration checks during the in-life phase of the study, the actual nominal test concentrations are 1.7, 0.87, 0.43, 0.22, 0.11 and 0.054 mg A.I./L plus the dilution water control.

Approval Signatures: Mark W. Machado — 11/3/92
Mark W. Machado, SLI Study Director — Date

Joseph W. Gorsuch — 11/13/92
Joseph W. Gorsuch, Sponsor Study Monitor — Date

APPENDIX 4 - FOOD AND DILUTION WATER ANALYSIS

#0292A7 Brine Shrimp Nauplii*		
Date Collected:3/3/92 Date Reported: 3/19/92		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0.01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg/kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	< 0.01 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCB's	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.05 mg/kg	0.05
Endosulfan I	< 0.01 mg/kg	0.01
Endosulfan II	< 0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
* Analyzed by Lancaster Laboratories, Inc.		

#0292A7 Brine Shrimp Nauplii*		
Date Collected: 3/3/92 Date Reported: 3/19/92		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I,II,III	attached	
Arsenic	1.7 ppm	0.1
Cadmium	< 0.2 ppm	0.2
Lead	< 0.2 ppm	0.2
Mercury	< 0.02 ppm	0 02
* Analyzed by Lancaster Laboratories, Inc.		

GFT Grab Water Sample*		
Date Collected:6/23/92 Date Reported: 7/9/92		
Analysis	Result As Received	Limit of Quantitation
Pesticide screen I;II;III	attached	
Mercury	< 0.0002 mg/l	0.0002
Arsenic	< 0.05 mg/l	0.05
Selenium	< 0.05 mg/l	0.05
Boron	< 0.05 mg/l	0.05
Thallium	< 0.1 mg/l	0.1
Aluminum	< 0.2 mg/l	0.2
Antimony	< 0.05 mg/l	0.05
Barium	< 0.2 mg/l	0.2
Beryllium	< 0.005 mg/l	0.005
Cadmium	< 0.005 mg/l	0.005
Calcium	7.4 mg/l	0.5
Chromium	< 0.05 mg/l	0.05
Cobalt	< 0.05 mg/l	0.05
Copper	< 0.02 mg/l	0.02
Iron	< 0.1 mg/l	0.1
Lead	< 0.05 mg/l	0.05
Magnesium	2.2 mg/l	0.5
Manganese	< 0.01 mg/l	0.01
Molybdenum	< 0.1 mg/l	0.1
Nickel	< 0.04 mg/l	0.04
Potassium	1.0 mg/l	0.5
Silver	< 0.01 mg/l	0.01
Sodium	13.3 mg/l	0.5
Titanium	< 0.05 mg/l	0.05
Vanadium	< 0.05 mg/l	0.05
Zinc	< 0.02 mg/l	0.02
* Analyzed by Lancaster Laboratories, Inc.		

GFT Grab Water Sample*		
Date Collected:6/23/92 Date reported: 7/9/92		
Analysis	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BBC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
* Analyzed by Lancaster Laboratories, Inc.		

APPENDIX 5 - ANALYTICAL METHODOLOGY

SUMMARY

The analytical procedure for crotonaldehyde consisted of derivatization and extraction followed by gas chromatography of the extract. Test and control solutions containing crotonaldehyde were derivatized with 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxamine HCl and sodium thiosulfate. Samples were then extracted once with hexane and an aliquot of the extract was analyzed on a gas chromatograph fitted with an electron capture detector (GC-ECD).

The analytical method was validated twice on separate days using diluent (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$) water samples fortified with crotonaldehyde at a concentration of 20.20 mg/mL. Samples were diluted as necessary prior to derivatization and extraction so that the final concentration in the extract would fall within the range of 1-10 mg/L. Recoveries of crotonaldehyde from the validation test samples averaged 88.5 ± 5.8%, with a limit of quantitation (LOQ) of
2.71×10^{-4} mg/mL. The mean recovery (standard deviation) was used to define limits for acceptance of Quality Control sample performance during ecotoxicology studies performed with crotonaldehyde. This range is established as three standard deviations from the mean recovery obtained during this method validation for crotonaldehyde, and was defined as 71.2 to 106%.

EXPERIMENTAL

Equipment

1. Instrument:	Hewlett Packard Gas Chromatograph Model 5890 equipped with a Hewlett Packard Model 7673A auto-sampler, Hewlett Packard Model Ni-63 electron capture detector and Hewlett Packard Model 3396A integrator.
2. Balance:	SP 182, four place analytical balance, ± 0.1 mg
3. Laboratory glassware:	syringes, volumetric pipets, volumetric flasks, graduated cylinders, test tubes, GC vials, and amber serum bottles.

Reagents

1. Hexane: reagent grade, Burdick & Jackson
2. Sodium sulfate: anhydrous
3. O-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl: Aldrich, 99+%, Lot # 03014MY
4. Sodium thiosulfate: Aldrich, 99+%, Lot # 04901JY

Test Material

Crotonaldehyde, Lot # 7-92, was received from Eastman Kodak Company, Rochester, New York on 23 July 1992 and was identified by the Study Sponsor to contain 93.8% active ingredient.

Instrumental Conditions

The gas chromatographic analysis was performed utilizing the following instrumental conditions:

Column:	DB-5, 30 m (length) x 0.319 mm I.D.
Gas flows:	Carrier gas - Helium, 3.33 mL/min.
	Make-up gas - Helium, 81.5 mL/min.
Temperatures:	injector - 230 °C
	Column - 100 to 250 °C ramp, 10 °C/minute,
	Detector - 300 °C
Injection Volume:	1 μL
Attenuation:	2^8
Threshold:	9
Peak Width:	0.04 minutes
Retention Time:	crotonaldehyde ≈ 6.8 min.

PROCEDURES

Preparation of Stock Solutions for the Analytical Standards

A new stock solution of crotonaldehyde was prepared on each of the two days the analytical method was validated. Solutions were prepared by weighing 0.1081 g (1st

validation) and 0.1083 (2nd validation) of the test material, which corresponded to approximately 0.100 g of active ingredient, into 100-mL volumetric flasks and diluting to volume with NANOpure® water. These stock solutions (1.01 mg/mL and 1.02 mg/mL) were used in the preparation of the analytical standards.

A new solution of the derivatizing reagent, 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxamine HCl, was prepared on each of the two days the analytical method was validated. Solutions were prepared by weighing 0.1015 g (1st validation) and 0.1016 g (2nd validation) of the derivatizing reagent into 100-mL volumetric flasks and diluting with NANOpure® water. The final concentration of the derivatizing reagent was 1.00 mg/mL.

Sample Fortification

Method validation/recovery samples were prepared on two occasions by weighing 2.1582 and 2.1580 g (2.02 gram as active ingredient) into 100 mL volumetric flasks and diluting to volume with ASTM Type II (NANOpure®) water. Triplicate aliquots (0.500 mL) were removed from these primary solutions (20.20 mg/mL) and diluted 4000X with diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$). An additional six diluent water samples were left unfortified and undiluted to be utilized as control samples.

Sampling Techniques

Sampling procedures typically include syphoning (using silicone tubing) from the midpoint of the test container (i.e., glass volumetric flasks, centrifuge tubes, or aquaria) into graduated cylinders for volumes greater than 100 mL, and pipetting (using volumetric pipets) from the midpoint of the test container for sample volumes less than or equal to 100 mL. Deviations from these practices, if any, are identified in the study report.

Derivatization and Extraction

To prepare the control solutions (reagent blanks), 1 mL of 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl was mixing in a test tube with 200 µL of 0.10 M sodium thiosulfate. After mixing, 10 mL of NANOpure® water were added and this mixture was allowed to stand at ambient temperature for 2 hours. In a similar manner, test samples were prepared by

mixing 1 mL of 0-(2,3,4,5,6-pentafluoro-benzyl) hydroxyamine HCl in a test tube with 200 μL of 0.10 M sodium thiosulfate. After mixing the derivatizing solution, 10 mL of each fortified sample were added to the derivitization mixture and allowed to stand at ambient temperature for two hours.

All samples (control and fortified) were then extracted by adding 1 - 3 drops of concentrated sulfuric acid to each test tube and mixed. A volume of 2 mL of hexane was added and the contents again shaken for 30 seconds. After allowing the test tube to stand for 15 minutes, the hexane was decanted from the aqueous solution and dried with sodium sulfate to remove any residual water. The sample was then transferred into a GC vial for analysis by gas chromatography (GC) using electron capture detection (ECD).

ANALYSIS

Preparation of Standards

A new set of standard solutions was prepared on each of the two days the analytical method was validated. The concentrations of crotonaldehyde in the standards were 10.1, 5.10, 2.53 and 1.01 mg/L (1st day) and 10.2, 5.10, 2.55, and 1.02 mg/L (2nd day). The standards were derivatized and extracted in the same manner as the samples. Injection of the samples and standards onto the chromatographic system was performed by programmed injection. Two complete sets of standards were analyzed with each sample set, one prior to the samples and one immediately following the samples.

CALCULATIONS

The following equations were used to calculate the measured concentrations of crotonaldehyde:

$$\frac{(\text{signal} - b)}{m} = DC$$

$$DC \times DF = A$$

where:

signal = summation of the two peak signals (heights) from chromatogram

b = y-intercept from regression analysis

m = slope from regression analysis

DC = detected concentration (mg/L) in the extract on GC

DF = dilution factor (final volume of the extract divided by the original aqueous volume extracted)

A = analytical result (mg/L), concentration in the original aqueous sample

The limit of quantitation (LOQ) was calculated using the following equation:

$$\frac{((0.5 \times A_{LS}) - b)}{m} = LOQ_{INST}$$

$$LOQ_{INST} \times DF_{CNTL} = LOQ$$

where:

A_{LS} = The mean signal response of the low concentration standard (two injections)

LOQ_{INST} = The minimum detected level on the instrument (extract)

DF_{CNTL} = The dilution factor of the control samples (smallest dilution factor used) = 1

LOQ = The minimum quantifiable level reported for samples regression analysis or point to point calibration (limit of quantitation)

RESULTS AND DISCUSSION

The mean recovery of crotonaldehyde in diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$) was 88.5 ± 5.8%, for samples with a nominal concentration of 20.20 mg/mL. The limit of quantitation for this method validation was 2.71 x 10^{-4} mg/mL. The LOQ may vary somewhat during subsequent analyses (ecotoxicology testing programs) since it is dependent upon the linear regression of the standards and the peak response (heights) of the low standards. These parameters, while relatively constant, do deviate somewhat and produce small variations in the LOQ. Recovery results from this method validation were used to evaluate Quality Control samples prepared during subsequent ecotoxicology studies

involving crotonaldehyde. Quality Control sample recovery expectations were three standard deviations from the mean recoveries obtained in method validation, 71.2 to 106%.

Analytical results for the recovery of crotonaldehyde from diluent water are presented in Table 1A. A representative chromatogram showing the analysis of derivatized crotonaldehyde in one of the standards is shown in Figure 1A. A representative chromatogram showing the analysis of derivatized crotonaldehyde from one of the fortified diluent water samples is shown in Figure 2A. The analysis of control water is presented in Figure 3A. A typical linear regression analysis for derivatized crotonaldehyde is presented in Figure 4A.

Table 1A. Analytical results for the recovery of crotonaldehyde from diluent water (fortified to a hardness of 160 - 180 mg/L as $CaCO_3$).

Fortified Concentration (mg/mL)	Volume Extracted (mL)	Recovered Concentration (mg/mL)	Percent Recovery[a] (%)
20.20	10.0	17.47	86.5
20.20	10.0	19.61	97.1
20.20	10.0	17.61	87.2
20.20	10.0	16.45	81.4
20.20	10.0	18.24	90.3
20.20	10.0	26.39	130.6[b]
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 2.71 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA
Control	10.0	$< 6.34 \times 10^{-4}$	NA

NA = Not Applicable

Mean recovery: 88.5 ± 5.8%, (N = 5).

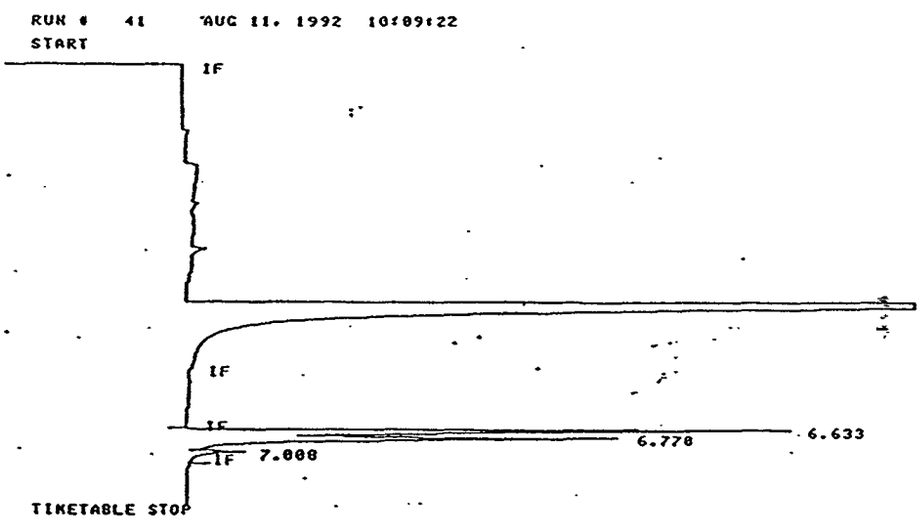
Limit of quantitation has been determined to be 2.71×10^{-4} mg/mL.

Values expressed as less than are below the limit of quantitation (LOQ). The LOQ for each sample is dependent upon the sample volume, dilution factor, and standard concentration range.

[a] Values presented are based on unrounded analytical results rather than the rounded values presented in this table.

[b] High percent recovery was determined to be an outlier using Chauvenet's Criterion and was not included in the calculation of the mean recovery.

Figure 1A. **Chromatogram of derivatized crotonaldehyde from one of the standards.**



RUN# 41 AUG 11, 1992 10:09:22

SAMPLE NAME: STD SAMPLE# 5
10 NG/L

IDENTIFIER : 2728A12338

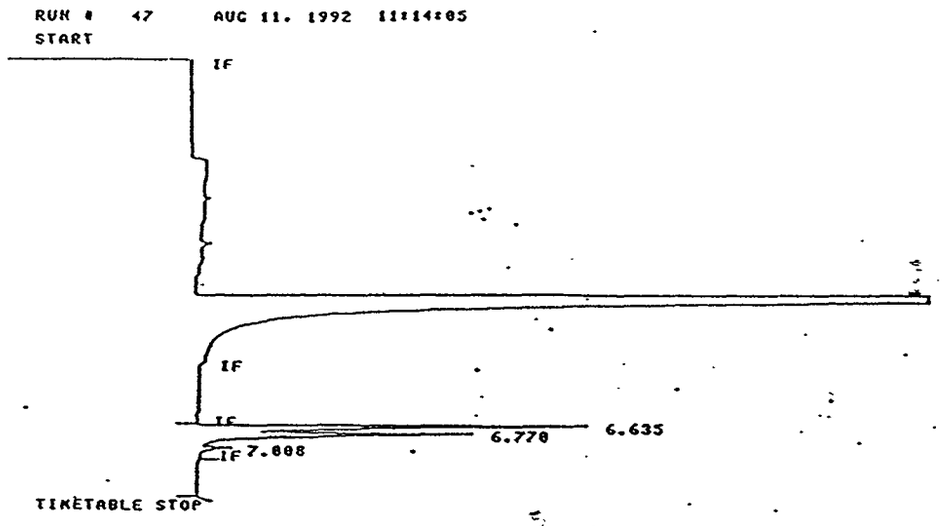
CROT

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	NG/L	NAME
6.888	++	10886888	.066	2732466	1R	5.503	CROT

TOTAL AREA=1.0887E+07
MUL FACTOR=1.0000E+00

Figure 2A. Chromatogram showing derivatized crotonaldehyde recoveries from one of the fortified samples.



RUN# 47 AUG 11, 1992 11:14:05

SAMPLE NAME: SK-C SAMPLE# 11

~~20000~~ NG/L
20200

IDENTIFIER : 2728A12358

CROT

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	NG/L	NAME
6.800	**	6779206	.865	1742420	IR	16136.492	CROT

TOTAL AREA=6779206
RUL FACTOR=5.0000E-03

Data replaced upon changing the S.I. of Std method
10/20/92 KT

Figure 3A. Chromatogram showing analysis of one of the control water samples.

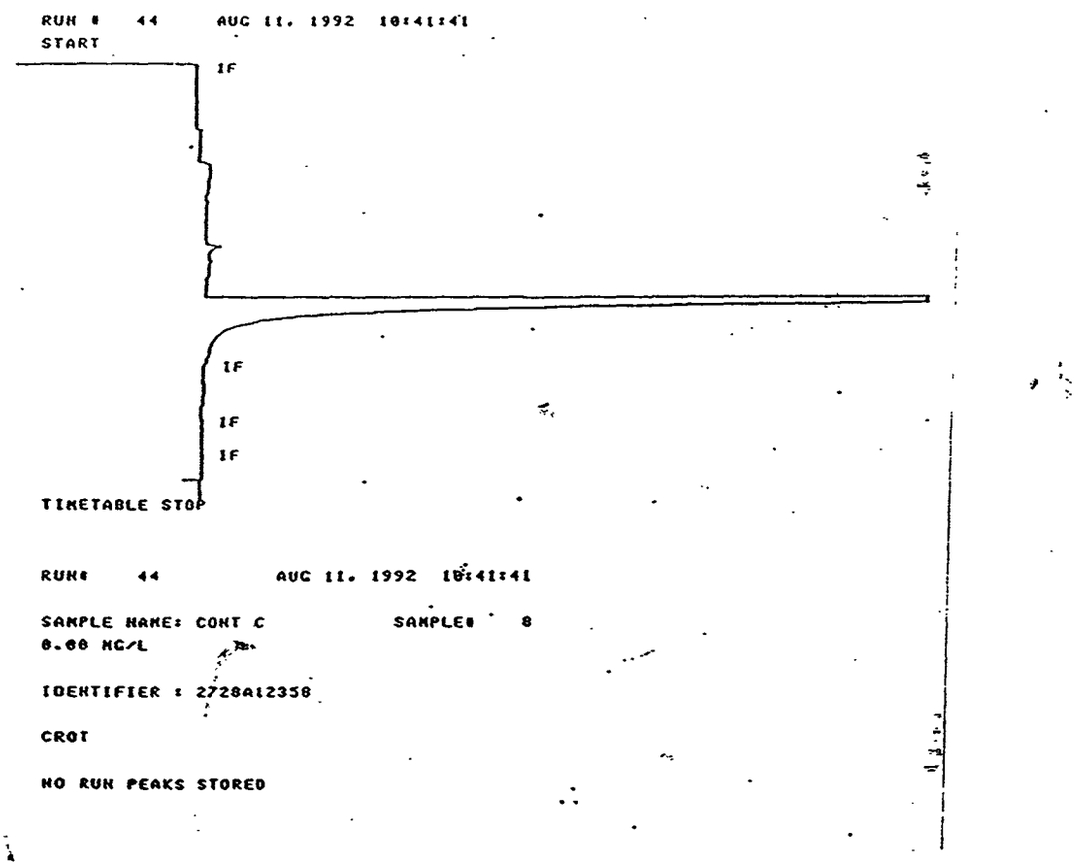
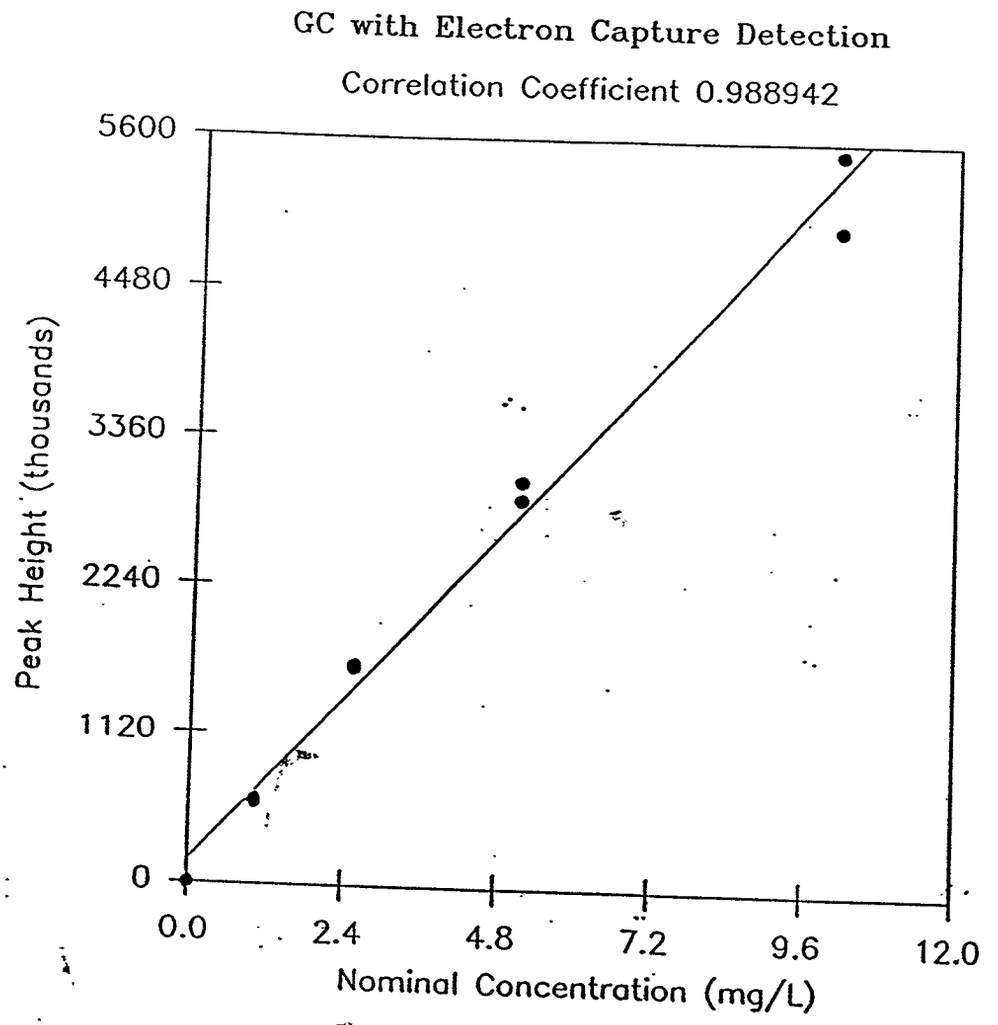


Figure 4A. **Plot of signal response versus concentration for derivatized crotonaldehyde linear regression analysis.**



APPENDIX 6 - CHEMICAL DISTRIBUTION RECORD

1852 0692 6103 130

page 36
SLI# 28-36

Test Material Log + Usage Book

Test Material: CROTONALDEHYDE Synonym
Received from: EASTMAN KODAK Co. City/State Rochester, NY 14650
Sponsor: EASTMAN KODACK City/State
Telephone #
Date received JULY 23, 1992 Date logged: JULY 23, 1992

Label information only:

Test Material CROTONALDEHYDE Net Wt. na
Lot, Batch, Code, I.D. Other # 7-92 Purity: na
Expiration Date na
Other Information: Storage: Under Nitrogen Tare Wt: 466.4 TOTAL WT: 1303.9g

Sponsor Information: Source by on
Test Material
Lot, Batch, Code, I.D. Other#: Purity:
as Salt as Base
CAS # [ ]
Molecular Wt: g/mole Solubility: (units)
Empirical Formula: Vapor Pressure:
Storage Conditions: Under nitrogen - TMC Refrig Dissociation Constant(s):
Other: NET WT.: ONE LITER

Radiolabelled:(only) Source na by na on na
Amount (mCi) na Sp.Activity na (units)
Radiochemical Purity: na Salt na Base na
Other

Characterization: By. Date
Color:
Solid Liquid Gas
Powder Viscous
Crystal
Pellet Other

(271)

Gross Wt. 1306.54g
Storage location:_____ Container: Amber Bottle

Hazard Rating: 3 by JMG on 6-18-92

Shipping Info:
Hazardous ✓ Non-Hazardous no
Classification: Flammable Liquid / Poison By Inhalation
DOT Label: Flammable Liquid + Poison
UN# 1143
Contact: Joe Gorsuch telephone # 716-588-2140

transcribed by pl on 7-23-92
verified by _____ on _____

Disposition of test material:
Returned to _____ by _____ on _____
Final Weight: _____

SPRINGBORN LABORATORIES, INC.

(A) This total was comprised of entries made 7/14/92 thru 7/26/92. These were recorded on the wrong SLI #. The total of 7.9375 will be deducted from the total used as of 9/28/92.

28-36

MOVED TO HSR

Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used	By	Study Number
7-28-92	1366.72			MS	1.3978	1.3978	NSP	1852.6103.130
7-28-92					0.1014	1.4992	JV	1852 6103-130
8-3-92					0.1015	1.6007	JV	1852 6103-250
8-4-92					0.1085	1.7092 (1) 2.6857	JV	1852 6103
8-7-92					0.1082	1.8174 2.7939	JV	1852-6103-250
8-7-92					2.1576	3.9750 6.0815	JV	1852-6103-250
8-7-92					0.1081	5.0896 6.1831	JV	1852-6103-250
8-7-92					2.1580	6.2411 8.2476	JV	1852-6103-250
8-10-92					0.1083	6.3494 8.3559	JV	1852-6103-250
8-10-92					2.1582	8.5076 10.5141	JV	1852-6103-250
8/11/92					5.5022	14.0098 19.5120	ROB	1852.6103.130
8-17-92					2.1579	16.1677 18.2741	JV	1852 6103-130
8-17-92					0.1080	16.2757 18.3822	JV	1852 6103-130
8-18-92					0.1080	16.3837 18.4902	JV	185-26103-130
8-18-92					2.1581	18.5418 20.6483	JV	185-26103-130
8-19-92					2.1585	20.7003 22.8068	JV	185-26103-130
8.20.92					2.2650	22.9653 25.2303	RR	1852.6103
* 7/14/92					0.9923	23.9576 (A) 24.9644	ECP	1852.6102.6102-120
* 7/16/92					0.9921	24.9497 25.9565		
* 7/18/92					0.9924	25.9421 26.9489		
* 7/18/92					0.9921	26.9343 27.9410		
* 7/20/92					0.9922	27.9264 28.9331		
* 7/22/92					0.9924	28.9188 29.9255		
* 7/24/92					0.9921	29.9109 30.9176		
* 7/26/92					0.9919	30.9028 31.8947		
* 7/28/92					4.9599	35.8627 36.8546		

* (LE) HEL 8/25/92

(1) Error in calculation MS 9-21-92

SPRINGBORN LABORATORIES, INC.

	Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used	By	Study Number
*	7/30/92					0.9924	36.8551 ~~37.8614~~ ①	PER	1852.0692.6102.110
*	8/4/92					~~3.5225~~ 3.5635	~~40.1186~~ 40.4186 ② ~~41.4251~~		
*	8/5/92					3.5620	43.9806 ② ~~44.9871~~		
*	8/6/92					3.5628	47.5434 ② ~~48.5499~~		
*	8/7/92					3.5620	51.1054 ② ~~52.1119~~		
*	8/8/92					3.5624	54.6678 ② ~~55.6743~~		
*	8/9/92					3.5625	58.2303 ② ~~59.2368~~		
*	8/10/92					3.5608	61.7911 ② ~~62.7976~~		
⊙	8/20/92					3.6577	65.4488 ② ~~66.4477~~	MOB	1852.0692.6103.130
⊙	8/22/92					3.6513	69.0994 ② ~~70.1001~~	MOB	1852.0692.6103.130
⊙	8/24/92					3.6515	72.7509 ② ~~73.7516~~	MOB	1852.0692.6103.130
	8/24/92					3.6523	76.3979 ② ~~77.4039~~	MOB	1852.0692.6103.130
	8-26-92					2.1584	78.5563 ② ~~79.6623~~	JV	1852-0692-6103
	8-26-92					0.1080	78.6643 ② ~~79.6703~~	JV	1852.0692-6103
*	8-25-92					0.1083	78.7726 ② ~~79.7786~~	JV	
*	8-25-92					2.1579	80.9305 ② ~~81.9365~~		
*	8-24-92					0.1083	81.0388 ② ~~82.0448~~		
*	8-24-92					2.1583	83.1971 ② ~~84.2031~~	JV	
	8-28-92					3.6522	86.8493 ② ~~87.8553~~	MOB	1852.0692.6103.130
	8-28-92					0.1083	86.9576 ② ~~87.9636~~	JV	1852-0692-6103
	8-31-92					0.1083	87.0659 ② ~~88.0719~~		
	8-31-92					2.1582	89.2241 ② ~~90.2301~~		
	9-5-92					3.6520g	93.8761 ② ~~93.8821~~	BAT	1852.0692.6103.130
	9-7-92					9.131g	103.0082 ② ~~103.0136g~~	BAT	1852.0692.6103.130
	9-7-92					0.1083	102.1154 ② ~~103.1214~~	JV	1852-0692-6103
	9-7-92					2.1581	104.2735 ② ~~105.2795~~	JV	1852-0692-6103

① Lenos 8/24/92 ② Error in Calculation ie MS 9-21-92

* ② Exel 8/25/92 (...)

SPRINGBORN LABORATORIES, INC.

SLI# _____

① Calculation Error MS 9-21-92

Date	Initial Weight	Final Weight	Difference	By	Actual Used	Total Used ①	By	Study Number
9-14-92				JV	0.1083	104.3818 ~~105.3883~~	JV	1852-6102
9-14-92				JV	2.1579	106.5397 ~~107.5462~~ ①	JV	1852-6102
9-14-92				MDB	9.1310	115.6707 ~~116.6772~~ ①	MDB	1852.0692.6103.130
9-3-92				MDB	3.6523	119.3230 ~~120.3295~~ ①	MDB	1852.0692.6103.130
9/21/92				MDB	9.1309	128.4539	MDB	1852.0692.6103.130
8-21-92				JV	0.1081	128.562	JV	1852-0692-6103
8-21-92				JV	2.1584	130.7204	JV	1852-0692-6103
9-21-92				JV	0.1079	130.8283	JV	1852-0692-6103
9-21-92				JV	2.1579	132.9862	JV	1852-0692-6103
② 8/30/92				WC	3.6521	136.6383	WC	1852.0692.6102.1
② 9/1/92				WC	3.6520	140.2903	WC	1852.0692.6102.1
9-28-92				JV	0.1081	140.3984	JV	1852-0692-6103
9-28-92				JV	2.1579	142.5563	JV	1952-0692-6103
10-5-92				JV	2.1579	144.7142	JV	1952-0692-6102
10-5-92				JV	0.1081	144.8223	JV	1952-06-92-610
9/28/92				MDB	9.1311	④ 146.0159 ~~153.9534~~	MDB	1852.0692.6103.130

Sample 1 was 11.23.92

Sample 1 was 11.23.92

APPENDIX 7 - STATISTICAL ANALYSES

MATC Program Methods and Calculations

Williams' Test is a parametric procedure considered to be preferable for chronic toxicity testing, but by design, assumes that the mean response of a variate is a monotonic function of concentration. Similar to Dunnett's Test, the Williams' test compares each of the group means to the control. However, it is used in a "step-down" manner (according to treatment levels) which enables the analysts to determine the concentration at which the monotonic function deteriorates, hence evidence for a significant response.

Representative Statistical Output

1852 0692 6102 120

200

TITLE: Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
FILE: KK
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9000	1.2490
1	Control	2	0.9000	1.2490
2	0.061	1	0.8750	1.2094
2	0.061	2	0.9250	1.2934
3	0.12	1	0.9000	1.2490
3	0.12	2	0.7000	0.9912
4	0.25	1	0.8250	1.1392
4	0.25	2	0.7750	1.0766
5	0.49	1	0.7750	1.0766
5	0.49	2	0.8500	1.1731
6	0.98	1	0.6500	0.9377
6	0.98	2	0.8000	1.1071
7	2.0	1	0.0000	0.0791
7	2.0	2	0.0000	0.0791

vc
10/14/92

1852 0692 6102 120 201

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: KK Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.058

W = 0.983

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

uc
10/14/92

1852 0692 6102 120 202

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: KK Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

WC
10/14/92

203

1852 0692 6102 1201

TITLE: Crotonaldehyde FHM ELS 1852-0692-6102-120 EMB SUR HATCH
FILE: CROFM.ESH
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	* 0.8999 ✓	1.2489
1	Control	2	0.9000 ✓	1.2490
2	0.061	1	0.8750 ✓	1.2094
2	0.061	2	0.9250 ✓	1.2934
3	0.12	1	0.9000 ✓	1.2490
3	0.12	2	0.7000 ✓	0.9912
4	0.25	1	0.8250 ✓	1.1392
4	0.25	2	0.7750 ✓	1.0766
5	0.49	1	0.7750 ✓	1.0766
5	0.49	2	0.8500 ✓	1.1731
6	0.98	1	0.6500 ✓	0.9377
6	0.98	2	0.8000 ✓	1.1071
7	2.0	1	0.0000 ✓	0.0791
7	2.0	2	* 0.0001 ✓	0.0100

* ACTUAL VALUES 0.0000 + 0.9000 BUT IN ORDER TO PASS BARTLETT'S TEST (MINUS) ARC VARIABILITY WAS ADDED.
9/14/92 MWT

ALL OTHER VALUES VERIFIED + CORRECT. MWT 9/17/92

WC 10/14/92

1852 0692 6102 120

264

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMB SUR HATCH
File: CROFM.ESH Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.060

W = 0.987

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

W CA 10/9/92

1852 0692 6102 1204

205

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMB SUR HATCH
File: CROFM.ESH Transform: ARC SINE(SQUARE ROOT(Y))

Bartletts test for homogeneity of variance

Calculated B statistic = 11.39
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

VC
10/9/92

206

TITLE: Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
FILE: YY
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9000	1.2490
1	Control	2	0.9000	1.2490
2	0.061	1	0.8750	1.2094
2	0.061	2	0.9250	1.2934
3	0.12	1	0.9000	1.2490
3	0.12	2	0.7000	0.9912
4	0.25	1	0.8250	1.1392
4	0.25	2	0.7750	1.0766
5	0.49	1	0.7750	1.0766
5	0.49	2	0.8500	1.1731
6	0.98	1	0.6500	0.9377
6	0.98	2	0.8000	1.1071
7	2.0	1	0.0000	0.0791
7	2.0	2	0.0000	0.0791

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Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	2	1.249	1.249	1.249
2	0.061	2	1.209	1.293	1.251
3	0.12	2	0.991	1.249	1.120
4	0.25	2	1.077	1.139	1.108
5	0.49	2	1.077	1.173	1.125
6	0.98	2	0.938	1.107	1.022
7	2.0	2	0.079	0.079	0.079

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.000	0.000	0.000
2	0.061	0.004	0.059	0.042
3	0.12	0.033	0.182	0.129
4	0.25	0.002	0.044	0.031
5	0.49	0.005	0.068	0.048
6	0.98	0.014	0.120	0.085
7	2.0	0.000	0.000	0.000

208

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
ile: YY Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
etween	6	2.030	0.338	42.250
Within (Error)	7	0.058	0.008	
otal	13	2.088		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F REJECT Ho:All groups equal

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Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1.249	0.900		
2	0.061	1.251	0.900	-0.026	
3	0.12	1.120	0.800	1.442	
4	0.25	1.108	0.800	1.578	
5	0.49	1.125	0.813	1.389	
6	0.98	1.022	0.725	2.533	
7	2.0	0.079	0.000	13.080	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	2			
2	0.061	2	0.195	21.6	-0.000
3	0.12	2	0.195	21.6	0.100
4	0.25	2	0.195	21.6	0.100
5	0.49	2	0.195	21.6	0.087
6	0.98	2	0.195	21.6	0.175
7	2.0	2	0.195	21.6	0.900

Crotonaldehyde PHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2.030	0.338	42.250
Within (Error)	7	0.058	0.008	
Total	13	2.088		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F REJECT Ho:All groups equal

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1.249	0.900		
2	0.061	1.251	0.900	-0.026	
3	0.12	1.120	0.800	1.442	
4	0.25	1.108	0.800	1.578	
5	0.49	1.125	0.813	1.389	
6	0.98	1.022	0.725	2.533	
7	2.0	0.079	0.000	13.080	*

Bonferroni T table value = 3.13 (1 Tailed Value, P=0.05, df=7,6)

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	2			
2	0.061	2	0.220	24.5	-0.000
3	0.12	2	0.220	24.5	0.100
4	0.25	2	0.220	24.5	0.100
5	0.49	2	0.220	24.5	0.087
6	0.98	2	0.220	24.5	0.175
7	2.0	2	0.220	24.5	0.900

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Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.900	1.249	1.250
2	0.061	2	0.900	1.251	1.250
3	0.12	2	0.800	1.120	1.120
4	0.25	2	0.800	1.108	1.116
5	0.49	2	0.813	1.125	1.116
6	0.98	2	0.725	1.022	1.022
7	2.0	2	0.000	0.079	0.079

Crotonaldehyde FHM ELS 1852-0692-6102-120 EMSUHA
File: YY Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	1.250				
0.061	1.250	0.013		1.89	k= 1, v= 7
0.12	1.120	1.420		2.00	k= 2, v= 7
0.25	1.116	1.461		2.04	k= 3, v= 7
0.49	1.116	1.461		2.06	k= 4, v= 7
0.98	1.022	2.495	*	2.07	k= 5, v= 7
2.0	0.079	12.880	*	2.08	k= 6, v= 7

= 0.091
Note: df used for table values are approximate when v > 20.

APPENDIX 8 - EXCERPTED RAW DATA

SPRINGBORN LABORATORIES, INC. Page 3D

RESULTS OF CHROMATOGRAPHIC ANALYSIS
TABLE OF MEANS

Sponsor: EASTMAN KODAK COMPANY
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6102-120
Test Type: EARLY LIFE STAGE W/FHM
Data Entered By: RT RT
Date Program Run: 04-Nov-92

Sample ID	Nominal Concentration (mg/L)	Analytical Result (mg/L)	Mean Analytical Result (mg/L)	N	Coeffi. of Varia.	day
9-92-67	17000	1.552E+04	18266	17	14.59	0
9-92-68	17000	1.624E+04				0
9-92-69	17000	1.763E+04				0
9-92-310	17000	2.285E+04				5
9-92-311	17000	2.141E+04				5
9-92-312	17000	2.245E+04				5
9-92-857	17000	2.004E+04				12
9-92-858	17000	1.867E+04				12
9-92-859	17000	2.020E+04				12
9-92-1379	17000	1.514E+04				19
9-92-1380	17000	1.419E+04				19
9-92-1381	17000	1.477E+04				19
9-92-1742	17000	1.918E+04				26
9-92-1743	17000	2.004E+04				26
9-92-1744	17000	1.976E+04				26
10-92-248	17000	1.624E+04				33
10-92-249	17000	1.619E+04				33

SPRINGBORN LABORATORIES, INC. Page 58

RESULTS OF CHROMATOGRAPHIC ANALYSIS
QA DATA SUMMARY

Sponsor: EASTMAN KODAK COMPANY
Test Material: CROTONALDEHYDE
Project No.: 1852-0692-6102-120
Test Type: EARLY LIFE STAGE W/FHM
Data Entered By: RT
Date Program Run: 20-Oct-92 Reprocessed data RT

Sample ID	Nominal Concentration (mg/L)	Y Evaluate (mg/L)	Analytical Result (mg/L)	Percent of Nominal	DAY
9-92-71 QA1	20200	4.206E+00	1.682E+04	83.3	0
9-92-314 QA1	20200	4.647E+00	1.859E+04	92	5
9-92-861 QA1	20200	5.376E+00	2.150E+04	106	12
9-92-1383 QA1	20200	4.518E+00	1.807E+04	89	19
9-92-1746 QA1	20200	4.900E+00	1.960E+04	97.0	26
10-92-245 QA1	20200	5.275E+00	2.110E+04	104	33
9-92-72 QA2	20200	4.416E+00	1.766E+04	87.4	0
9-92-315 QA2	20200	4.625E+00	1.850E+04	92	5
9-92-862 QA2	20200	5.551E+00	2.221E+04	110 *	12
9-92-1384 QA2	20200	4.172E+00	1.669E+04	83	19
9-92-1747 QA2	20200	4.953E+00	1.981E+04	98.1	26
10-92-246 QA2	20200	5.050E+00	2.020E+04	100	33
9-92-73 QA3	20200	4.726E+00	1.890E+04	94	0
9-92-316 QA3	20200	4.713E+00	1.885E+04	93.3	5
9-92-863 QA3	20200	5.497E+00	2.199E+04	109 *	12
9-92-1385 QA3	20200	4.622E+00	1.849E+04	92	19
9-92-1748 QA3	20200	4.877E+00	1.951E+04	96.6	26
10-92-247 QA3	20200	5.000E+00	2.000E+04	99.0	33

Mean Recovery : 94.2

standard deviation : 6.74

* These QA's are outside the 3rd standard deviation range established during the recovery study. It, therefore, is not used in the calculation.

% HEALTHY, FERTILE EMBRYOS @ 40-48 HRS

~~* EMBRYO SURVIVAL AT THE COMPLETION OF HATCH~~ : FATHEAD MINNOW

TEST DAY 2 DATA BY MWM DATE 9/11/92

(VA) 9/11/92

CONC. mg/L	EGG CUP	TOTAL # DEAD EGGS	DEAD FRY UH *	DEAD FRY SH *	TOTAL # DEAD FRY	LIVE DEFRMD. FRY	TOTAL LIVE # ~~FRY~~ EGGS	TOTAL NUMBER EXPOSED	NUMBER UNACCNT. FOR	PERCENT (%) LIVE ~~HATCH~~	ARC SIN % TRANSFRM		
2.0	1A	36					4	40	0	10.00			
	1B	40					0	40	0	0.00			
0.98	2A	6					34	40	0	85.00			
	2B	5					35	40	0	87.50			
0.49	3A	6					34	40	0	85.00			
	3B	5					35	40	0	87.50			
0.25	4A	4			(D) 9/10/92 MWM		36	40	0	90.00			
	4B	10					26	40	0	75.00			
0.12	5A	4					36	40	0	90.00			
	5B	9					31	40	0	77.50			
0.061	6A	4					35	40	0	87.50			
	6B	1					39	40	0	97.50			
Control	7A	1					39	40	0	97.50			
	7B	3					37	40	0	92.50			
(D) A	B	C	D	E	F	G	H	I	J	K	L	M	N

4/4 VA 9/10/92

* FOR THIS DETERMINATION A DEAD EGG IS CONSIDERED TO BE NON-FERTILE. 9/4/92 MWM

(D) SEE BACK FOR KEY

/89

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1852 0692 6102 120

EMBRYO SURVIVAL AT THE COMPLETION OF HATCH : FATHEAD MINNOW

TEST DAY 5 (DAY 0 POST-HATCH)

DATA BY MWM

DATE 9/8/92

NOTE: ABSOLUTELY NO ABNORMAL/DEFORMED FRY NOTED. MWM 9/8/92

CONC. mg/L	EGG CUP	TOTAL DEAD EGGS	DEAD FRY UH ①	DEAD FRY SH ①	TOTAL # DEAD FRY	LIVE DEFRMD. FRY	TOTAL LIVE FRY	TOTAL NUMBER EXPOSED	NUMBER UNACCNT. FOR	PERCENT (%) HATCH	SRC SIN & TRANSFRM		
	1A	40	0	0	0	0	0	40	0	0.00			
2.0	1B	40	0	0	0	0	0	40	0	0.00			
	2A	8	3	3	6	0	26	40	0	65.00			
0.98	2B	6	0	2	2	0	32	40	0	80.00			
	3A	9	0	0	0	0	31	40	0	77.50			
0.49	3B	6	0	0	0	0	34	40	0	85.00			
	4A	5	2	0	0	0	33	40	0	82.50			
0.25	4B	9	0	0	0	0	31	40	0	77.50			
	5A	4	0	0	0	0	36	40	0	90.00			
0.12	5B	11	0	1	1	0	28	40	0	70.00			
	6A	5	0	0	0	0	35	40	0	87.50			
0.061	6B	1	1	1	2	0	37	40	0	92.50			
	7A	3	1	0	1	0	36	40	0	90.00			
CONTROL	7B	3	1	0	1	0	36	40	0	90.00			
A	B	C	D	E	F	G	H	I	J	K	L	M	N

② ③ CORRECTED WITH MWM

TEST DAY 5 WAS DETERMINED TO BE DAY 0 POST-HATCH ... THE RANGE OF TIME-TO-HATCH FOR ALL TREATMENTS LEVELS AND CONTROLS WAS DETERMINED TO BE THE SAME (i.e. 5 DAYS) BASED ON OBSERVATIONS ON THE ~~FOLLOWING PAGE~~ THE RECORD OF EGG INCUBATION PAGE AND THIS DETERMINATION (ABOVE).

MWM 9/8/92

① KEY TO ABOVE CHART FOUND FOLLOWING STATS MWM 9/21/92

MWM 9/21/92 ④

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1852 0692 6102 1200

LARVAL SURVIVAL AT TEST TERMINATION

DATE 10/6/92

DATA BY uc

CONCENTRATION (mg/l)	REPLICATE	NUMBER OF LARVAE INITIALLY EXPOSED	NUMBER OF LARVAE SURVIVING	PERCENT SURVIVAL	ARC SIN $\sqrt{x}$
2.0	1A	0	NA		
	1B	0	NA		
0.98	2A	29	23	79.31	
	2B	29	15	51.72	
0.49	3A	32	27	84.38	
	3B	33	28	84.85	
0.25	4A	32	24	75.00	
	4B	32	20	62.50	
0.12	5A	32	26	81.25	
	5B	32	21	65.63	
0.061	6A	36	30	83.33	
	6B	36	30	83.33	
CONTROL	7A	36	31	86.11	
	7B	36	28	77.78	

NA uc 10/9/92

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Summary of the Weekly Water Quality Analysis for the Early Life Stage Study with Crotonaldehyde

HARDNESS

	Control	0.061	0.12	0.25	0.49	0.98	2.0
Mean	~~29~~ 30	31	NA	NA	NA	NA	~~30~~ 31
STD	~~2.4~~ 2.5	3.0	NA	NA	NA	NA	~~3.1~~ 3.3
Range	26-32	26-34	NA	NA	NA	NA	~~26-34~~ 26-34
(N)	~~7~~ 6	~~7~~ 6	NA	NA	NA	NA	~~7~~ 6

ALKALINITY

Mean	22	23	NA	NA	NA	NA	~~23~~ 24
STD	~~3.3~~ 3.9	~~2.3~~ 2.4	NA	NA	NA	NA	~~1.9~~ 2.0
Range	16-26	20-26	NA	NA	NA	NA	22-26
(N)	~~7~~ 6	~~7~~ 6	NA	NA	NA	NA	~~7~~ 6

CONDUCTIVITY

Mean	140	140	NA	NA	NA	NA	~~130~~ 140
STD	~~13~~ 8.2	~~13~~ 8.2	NA	NA	NA	NA	~~9.0~~ 5.2
Range	~~130-150~~	~~130-150~~	NA	NA	NA	NA	~~130-150~~ 130-140
(N)	~~8~~ 6	~~8~~ 6	NA	NA	NA	NA	~~8~~ 6

ACIDITY

Mean	~~7.8~~ 6.5	~~6.6~~ 6.0	NA	NA	NA	NA	~~7.1~~ 6.3
STD	~~2.4~~ 1.4	~~2.1~~ 1.5	NA	NA	NA	NA	~~3.7~~ 3.3
Range	~~5-12~~ 5-8	~~5-10~~ 5-8	NA	NA	NA	NA	~~4-12~~ 4-11
(N)	~~7~~ 6	~~7~~ 6	NA	NA	NA	NA	~~7~~ 6

(1) VALUES RE-CALCULATED DUE TO ERROR IN # of 'N' VALUES, STUDY INITIATED ON 9/2/93, THUS N=6.
MWH 11/5/92

WC 10/14/92

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Summary of the Monthly Water Quality Analysis
of the GFT Dilution Water

	August	September	October
Tot. Suspended Solids	≤ 4 mg/L	≤ 4 mg/L	≤ 4 mg/L
Chlorine Residual	≤ 0.05 mg/L	≤ 0.05 mg/L	< 0.05 mg/L
Tot. Organic Carbon	0.9 mg/L	0.8 mg/L	0.5 mg/L
Chem. Oxygen Demand	≤ 5 mg/L	≤ 7 mg/L	≤ 7 mg/L
Ammonia (N)	≤ 0.1 mg/L [a]	≤ 0.1 mg/L	≤ 0.1 mg/L

Note: Monthly measurements of water quality parameters made by Lancaster Labs.

a = Measurement made by SLI staff, measured as Total Ammonia Nitrogen.

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Summary of the Water Quality Analysis of the Test Solutions

Total Organic Carbon (mg/L)

Date	Control	0.061	2.0
9/2	1.382	1.873	2.508
9/9	7.032	4.313	4.240
9/16	1.288	1.762	1.493
9/23	8.116	5.957	5.751
9/30	1.485	1.607	1.987
10/5	1.160	1.226	1.492
Mean	3.41	2.79	2.91
STD	3.24	1.90	1.72
(n)	6	6	6
Range	1.160-8.116	1.226-5.957	1.492-5.751

Total Suspended Solids (mg/L)

Date	Control	0.061	2.0
9/2	2.0	2.5	1.6
9/9	17.4	12.2	6.9
9/16	0	2.5	1.2
9/23	4.7	3.8	5.0
9/30	2.1	3.7	1.8
10/5	8.0	5.1	4.0
Mean	5.70	4.97	3.42
STD	6.36	3.67	2.27
(n)	6	6	6
Range	0-17.4	2.5-12.2	1.2-6.9

Un-ionized Ammonia (µg/L)

(†) PRESERVED AND SENT TO LANCASTER LABS

Date	Control	Date	Control
9/3	① ≤ 0.1 mg/L	9/28 (†)	6.2 mg/L (1.1 mg/L AS UN-IONIZED)
9/8	≤ 0.1 mg/L *	10/1 (†)	↓
9/10	≤ 0.1 mg/L *	10/5 (†)	
9/14	≤ 0.1 mg/L *		
9/17	≤ 0.1 mg/L *		
9/21	≤ 0.1 mg/L		
9/24	≤ 0.1 mg/L		

MEAN = 0.701 mg/L AS UN-IONIZED
S.D. = 0.28

* Due to instumentation malfunction these samples were taken and stored refrigerated until analysis could be performed. Samples were not acidified.

(REFER ALSO TO p.341) MWM 10/7/92
† p.342

① 0.1 mg/L TOTAL = 0.53 mg/L (ppb) AS UN-IONIZED 11/5/92 MWM

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Crotonaldehyde FHM ELS 1852-0692-6102-120 Larval Length (Total Length)

Conc. (ppb)	CONTROL		0.061		0.12		0.25		0.49		0.98	
Rep.	A	B	A	B	A	B	A	B	A	B	A	B
	30	32	30	30	27	27	26	30	29	26	22	24
	23	31	32	27	29	28	28	29	30	27	24	24
	29	31	30	30	26	29	26	32	27	27	23	25
	28	29	31	31	29	29	29	32	26	28	28	24
	31	29	29	28	32	31	32	27	29	26	24	23
	26	30	30	30	29	32	29	31	29	26	27	25
	26	32	30	30	28	31	28	27	29	27	26	26
	31	29	29	25	29	27	29	27	28	28	24	25
	33	28	25	33	32	31	29	29	32	25	25	25
	30	27	30	26	28	29	27	27	31	26	24	21
	32	33	32	32	27	28	32	25	31	28	27	24
	32	28	29	30	31	30	28	29	23	27	24	30
	33	30	29	30	30	28	26	27	24	29	27	20
	28	28	32	31	28	32	30	29	26	29	25	24
	35	31	29	33	27	27	24	30	28	26	22	28
	29	27	31	31	30	30	29	27	23	28	24	
	28	22	24	29	23	32	24	28	26	29	24	
	30	30	28	28	28	28	28	27	29	28	20	
	32	31	30	32	27	28	27	28	24	26	26	
	26	31	30	28	27	31	31	31	28	28	26	
	31	30	30	29	28	30	29		29	25	26	
	27	31	34	30	28		26		29	28	26	
	32	31	30	33	26		28		28	30	24	
	30	23	31	31	32		30		29	26		
	30	31	26	32	28				29	29		
	33	26	28	30	29				28	28		
	27	31	31	28					27	27		
	29	28	28	30						17		
	24		31	17								
	31		20	16								
	29											
MEAN	29.51612	29.28571	29.3	29	28.38461	29.42857	28.125	28.6	27.81481	26.92857	24.69565	24.53333
STD DEV.	2.779340	2.565542	2.705677	3.921646	2.041115	1.719634	2.132818	1.930366	2.337602	2.340126	1.893404	2.416215
N	31	28	30	30	26	21	24	20	27	28	23	15
MIN	23	22	20	16	23	27	24	25	23	17	20	20
MAX	35	33	34	33	32	32	32	32	32	30	28	30
COV	0.094163	0.087603	0.092343	0.135229	0.071909	0.058434	0.075833	0.067495	0.084041	0.086901	0.076669	0.098487

MEAN(A&B)		29.40677		29.15		28.85106		28.34090		27.36363		24.63157
STD. DEV.		2.659521		3.343726		1.955802		2.033935		2.359877		2.084966
N		59		60		47		44		55		38
MIN		22		16		23		24		17		20
MAX		35		34		32		32		32		30

Data verified
ve
10/8/92

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Crotonaldehyde FHM ELS 1852-0692-6102-120 Larval Weight

Conc. (ppb)	CONTROL		0.061		0.12		0.25		0.49		0.98	
Rep.	A	B	A	B	A	B	A	B	A	B	A	B
	0.1833	0.3753	0.2836	0.1767	0.2081	0.4110	0.2068	0.2545	0.2240	0.1862	0.0933	0.1393
	0.1635	0.2644	0.3221	0.1772	0.2485	0.2575	0.1897	0.2612	0.2786	0.2195	0.1517	0.1427
	0.2125	0.3330	0.1802	0.2869	0.2095	0.2719	0.2011	0.3289	0.2048	0.2011	0.1140	0.1700
	0.1560	0.2931	0.2991	0.2981	0.2745	0.3438	0.2777	0.3346	0.2053	0.2413	0.2317	0.1347
	0.2450	0.2878	0.2660	0.2541	0.3680	0.3361	0.3156	0.1776	0.2579	0.1905	0.1495	0.1146
	0.1816	0.2816	0.3065	0.2707	0.2982	0.2693	0.2323	0.3126	0.2727	0.2089	0.2108	0.1707
	0.1282	0.3270	0.2663	0.3356	0.2651	0.2852	0.2374	0.1994	0.2443	0.2172	0.1888	0.2000
	0.3167	0.3111	0.2434	0.1433	0.3009	0.2175	0.2481	0.1795	0.2597	0.2287	0.1511	0.1926
	0.2434	0.2299	0.1264	0.3819	0.3446	0.3356	0.2658	0.2473	0.4238	0.1687	0.1700	0.2006
	0.2669	0.1830	0.2762	0.1737	0.2433	0.1830	0.2162	0.2131	0.3135	0.1953	0.1564	0.0969
	0.2661	0.3734	0.3312	0.3021	0.1909	0.2519	0.3526	0.1596	0.3138	0.2270	0.2119	0.1622
	0.2716	0.1981	0.3005	0.3233	0.2287	0.2583	0.2176	0.2702	0.1229	0.2519	0.1747	0.2642
	0.3432	0.2521	0.2521	0.2865	0.2770	0.2448	0.2110	0.2161	0.1895	0.3046	0.2401	0.0768
	0.2070	0.1928	0.3903	0.2805	0.2366	0.3271	0.3012	0.2393	0.1639	0.2483	0.1668	0.1720
	0.3673	0.3324	0.2482	0.3844	0.2587	0.2103	0.1299	0.3084	0.2304	0.1980	0.1170	0.2480
	0.2650	0.1907	0.2920	0.3040	0.2457	0.3400	0.2410	0.2206	0.1553	0.2487	0.1413	
	0.1931	0.1437	0.1841	0.2691	0.1880	0.3929	0.1294	0.2554	0.1747	0.2684	0.1745	
	0.2286	0.3033	0.2313	0.2401	0.1316	0.2691	0.2285	0.2077	0.2570	0.2482	0.0975	
	0.3006	0.3284	0.2771	0.3339	0.1600	0.2480	0.2087	0.2390	0.1626	0.1855	0.1981	
	0.1653	0.3246	0.2580	0.1730	0.1967	0.2957	0.3025	0.2984	0.2369	0.2233	0.1728	
	0.2695	0.2564	0.2875	0.2694	0.2017	0.2876	0.2520		0.2710	0.1560	0.1854	
	0.1749	0.2903	0.4110	0.2557	0.2181		0.1878		0.3253	0.2122	0.2258	
	0.2893	0.3639	0.2429	0.3674	0.1549		0.2112		0.2589	0.3275	0.1399	
	0.2597	0.1193	0.3075	0.2988	0.1993		0.2988		0.2399	0.1942		
	0.2493	0.2961	0.1598	0.3107	0.2703				0.2504	0.2675		
	0.3045	0.1722	0.2127	0.3143	0.3162				0.2229	0.2354		
	0.1661	0.2633	0.3062	0.2483					0.2211	0.2207		
	0.2494	0.2385	0.2296	0.2773						0.0549		
	0.1617		0.2697	0.0478								
	0.2644		0.0541	0.0173								
	0.2169											
MEAN	0.235825	0.268775	0.26052	0.26007	0.239811	0.287457	0.235954	0.24617	0.240040	0.218917	0.167960	0.165686
STD DEV.	0.059359	0.069209	0.071595	0.086824	0.057574	0.058180	0.054193	0.051142	0.061968	0.049829	0.040849	0.051494
N	31	28	30	30	26	21	24	20	27	28	23	35
MIN	0.1282	0.1193	0.0541	0.0173	0.1316	0.183	0.1294	0.1596	0.1229	0.0549	0.0933	0.0768
MAX	0.3673	0.3753	0.411	0.3844	0.368	0.411	0.3526	0.3346	0.4238	0.3275	0.2401	0.2642
COV	0.251709	0.257501	0.274818	0.333851	0.240084	0.202398	0.229677	0.207752	0.258157	0.227619	0.243208	0.310795
MEAN(A&B)		0.251462		0.260295		0.2611		0.240597		0.229287		0.167063
STD. DEV.		0.065785		0.078898		0.062021		0.052470		0.056603		0.044685
N		59		60		47		44		55		38
MIN		0.1193		0.0173		0.1316		0.1294		0.0549		0.0768
MAX		0.3753		0.411		0.411		0.3526		0.4238		0.2642

Data verified
WC
10/8/92

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Dissolved Oxygen Values Crotonaldehyde FHM ELS 1852-0692-6102-120

Conc. (mg/L)	Control		0.061		0.12		0.25		0.49		0.98		2.0	
Rep.	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	8.8	8.8	8.7	8.8	8.7	8.7	8.7	8.8	8.7	8.7	8.7	8.7	8.7	8.7
	8.6	8.5	8.3	8.5	8.4	8.5	8.5	8.5	8.5	8.4	8.4	8.4	8.5	8.4
	8.7	8.5	8.6	8.6	8.5	8.5	8.5	8.5	8.5	8.5	8.4	8.5	8.4	8.4
	8.9	8.6	8.7	8.6	8.7	8.6	8.7	8.6	8.7	8.6	8.6	8.5	8.5	8.5
	8.9	8.6	8.7	8.7	8.7	8.6	8.7	8.7	8.7	8.7	8.6	8.6	8.5	8.5
	9.1	8.7	8.7	8.8	8.7	8.7	8.8	8.8	8.7	8.7	8.6	8.6	8.5	8.5
	8.7	8.4	8.3	8.6	8.6	8.5	8.6	8.4	8.7	8.3	8.4	8.3	8.5	8.4
	8.2	7.6	7.8	7.8	8.0	7.8	8.2	8.0	8.0	7.8	7.6	7.8	8.0	8.0
	8.7	8.2	8.4	8.4	8.4	8.4	8.4	8.5	8.3	8.3	8.2	8.0	8.3	8.6
	8.6	8.3	8.4	8.4	8.4	8.3	8.3	8.2	8.2	7.8	8.1	8.5	8.4	8.8
	8.3	8.1	7.9	8.1	8.2	7.8	8.0	8.1	8.0	8.0	7.6	7.4	8.0	8.8
	8.5	8.2	8.2	8.2	8.2	8.1	7.8	8.3	8.0	8.2	7.9	7.5	8.8	8.7
	8.1	8.0	8.4	7.9	8.3	8.1	8.3	8.0	7.9	8.3	8.1	8.0	8.6	8.5
	8.6	8.6	8.5	8.6	8.6	8.6	8.6	8.7	8.5	8.6	8.3	8.2	8.9	8.8
	8.2	8.0	7.9	8.0	8.0	8.1	8.1	8.3	8.1	7.9	7.9	7.6	8.7	8.5
	8.3	7.9	7.9	7.9	8.2	8.2	7.9	8.2	8.1	7.9	7.9	7.5	8.7	8.7
	8.2	8.1	7.9	8.1	8.1	8.2	8.1	8.4	8.2	7.8	8.2	7.8	8.6	8.5
	8.2	8.1	8.2	8.2	8.0	8.2	8.0	8.1	8.0	8.1	8.2	8.0	8.4	8.4
	8.0	7.9	7.8	7.8	7.9	8.0	7.9	8.0	7.8	8.0	7.8	7.9	8.2	8.3
	7.7	7.7	7.6	7.7	7.8	7.9	7.7	7.8	8.0	7.6	8.0	7.5	8.6	8.1
	7.8	7.9	7.4	7.6	7.6	7.5	7.5	7.4	7.5	7.5	7.8	7.2	8.6	8.6
	8.9	8.8	8.1	8.2	8.2	8.3	8.0	8.7	8.2	8.2	8.5	7.8	9.4	9.4
	8.8	8.8	8.1	8.3	8.2	8.1	8.1	8.4	8.1	8.2	8.5	7.9	9.6	9.6
	8.5	8.3	7.7	7.7	7.8	7.7	7.5	8.0	7.7	7.8	7.9	7.5	9.4	8.9
	11.0	10.8	10.0	10.2	9.6	10.0	9.6	10.4	9.9	10.0	10.0	9.6	11.0	10.2
	12.0	12.0	11.6	11.6	11.2	11.2	11.4	11.6	11.6	11.4	11.4	11.0	11.8	10.6
	8.5	8.9	8.1	8.5	7.9	7.9	7.7	8.1	8.3	8.2	8.1	8.3	8.9	8.5
	8.7	8.5	8.2	8.1	8.1	8.2	8.2	8.5	8.2	8.2	8.2	7.9	9.5	8.7
	9.2	9.3	8.9	9.0	8.7	8.7	8.8	9.0	8.7	8.7	8.9	8.5	10.0	10.0
	10.2	10.2	10.0	10.3	9.8	9.9	9.8	10.1	9.8	10.2	10.1	9.9	11.0	11.0
	8.2	8.3	7.3	7.8	7.5	7.8	7.7	8.1	7.6	8.3	7.7	7.6	9.2	9.5
	8.0	8.2	7.6	7.9	7.6	7.9	7.5	8.0	7.6	7.8	7.8	7.7	8.8	9.2
	7.8	7.8	7.7	7.6	7.8	7.5	7.6	7.7	7.6	7.7	7.7	7.6	8.6	8.8
	10.6	10.2	9.3	10.2	9.2	9.7	10.0	10.0	10.2	9.8	10.0	9.6	10.2	9.8
Mean	8.75	8.61	8.38	8.49	8.40	8.42	8.39	8.56	8.43	8.42	8.41	8.22	8.99	8.91
Std.Dev	0.92	0.93	0.84	0.88	0.72	0.77	0.81	0.83	0.84	0.82	0.82	0.81	0.89	0.71
N	34	34	34	34	34	34	34	34	34	34	34	34	34	34
Minimum	7.7	7.6	7.3	7.6	7.5	7.5	7.5	7.4	7.5	7.5	7.6	7.2	8	8
Maximum	12	12	11.6	11.6	11.2	11.2	11.4	11.6	11.6	11.4	11.4	11	11.8	11
Mean(A+B)		8.68		8.44		8.41		8.47		8.42		8.32		8.95
Std.Dev.		0.92		0.86		0.74		0.82		0.82		0.82		0.80
N		68		68		68		68		68		68		68
Minimum		7.6		7.3		7.5		7.4		7.5		7.2		8
Maximum		12		11.6		11.2		11.6		11.6		11.4		11.8

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Summary of the Daily Water Quality Analysis for the Early Life Stage Study with Crotonaldehyde

pH

	Control	0.061	0.12	0.25	0.49	0.98	2.0
Mean	NA	NA	NA	NA	NA	NA	NA
STD	NA	NA	NA	NA	NA	NA	NA
Range	6.9-7.5	6.8-7.3	6.8-7.3	6.8-7.3	6.8-7.3	6.8-7.3	6.8-7.4
(N)	68	68	68	68	68	68	68

Temperature

Mean	24	24	24	24	24	24	24
STD	0	0	0	0	0	0	0
Range	NA	NA	NA	NA	NA	NA	NA
(N)	68	68	68	68	68	68	68

Dissolved Oxygen

Mean	8.7	8.4	8.4	8.5	8.4	8.3	9.0
STD	0.92	0.86	0.74	0.82	0.82	0.82	0.80
Range	7.6-12	7.3-11.6	7.5-11.2	7.4-11.6	7.5-11.6	7.2-11.4	8.0-11.0
(N)	68	68	68	68	68	68	68

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